The Journal
of
Animal Morphology and Physiology

VOLUME VIII] DECEMBER 1961 [NUMBER 2

M. S. University Dept. of Zoology
FACULTY OF SCIENCE
BARODA (India)

The Journal of Animal Morphology and Physiology

This journal (*J. Anim. Morph. Physiol.*) published in two issues per year —one in June and the other in December, is the organ of the Society of Animal Morphologists and Physiologists and is issued free to all members of the Society.

Rates of Subscription

	India	U.S.A.	Other countries
Yearly for two issues.....	Rs. 13.00	$3.00	£1-0-0
For one issue	Rs. 7.00	$1.50	£0-10-0

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ANNOUNCEMENTS

The Second International Congress of Radiation Research will be held at Harrogate, Yorkshire, England, August 5th–11th, 1962. It is sponsored by a committee set-up at the First Congress at Burlington, Vermont, in 1958, and by the Association for Radiation Research. The programme will be concerned with the physical, chemical, biological and medical effects of radiations, particularly ionizing radiations. Research workers in these fields will be invited to proffer original papers and reports of new experimental work. A brochure will be available in April 1961. Information may be obtained from Dr. Alma Howard, Secretary-General, The Second International Congress of Radiation Research, Mount Vernon Hospital, Northwood, Middlesex, England.

THE XIIITH INTERNATIONAL ORNITHOLOGICAL CONGRESS

The XIIIth International Ornithological Congress will be held at Cornell University, Ithaca, New York, from 17 to 21 June 1962, under the presidentship of Prof. Ernst Mayr. General Secretary, Prof. C. G. Sibley, Fernow Hall, Cornell University, Ithaca, New York, U. S. A.

NOTICE

Owing to the rise in the cost of material and printing charges we are compelled to make a slight increase in the price of the Journal with effect from 1962. The new rates are as follows:

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MANAGING EDITOR,
J. Anim. Morph. Physiol.

STUDIES ON THE EXTERNAL MORPHOLOGY OF *PEREGRINUS MAIDIS* (ASHMEAD)

(Homoptera, Fulgoroidea, Araeopidae = Delphacidae) Part II, Thorax

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IN Part I, the external morphology of the head capsule and mouth parts of *Peregrinus maidis* has been described. The present paper deals with the morphology of the thoracic region. Crampton (1909), Snodgrass (1909) and Martin (1919) have worked out the structure of the thorax of generalised insects. Myers (1928), Hamilton (1931), Qadri and Aziz (1950) and Akbar (1957) have studied the thorax of various Hemipterous insects.

The thorax of *Peregrinus maidis* is well-developed and is divisible into an anterior prothoracic and a posterior pterothoracic region. The thin intersegmental membrane between the pro and pterothorax is especially enlarged at the sternal region. The pterothorax is formed by the union of meso and metathorax. The intersegmental membrane separating them has completely disappeared. Each segment bears a pair of legs and the second and third in addition possess a pair of wings.

Cervix Description

The cervix or neck is a narrow membranous area connecting the head with the thorax. It is stretched between the rim of the foramen magnum and the anterior region of the prothorax. It is concealed under the overlapping anterior region of the pronotum. The neck membrane is strengthened by a pair of lateral cervical sclerites. The anterior pair is developed in the lateral angles of the rim of the foramen magnum. It is hardly discernible. The posterior pair is curved and is located at the anterior margin of the episternum of prothoracic pleura. It simply rests on the anterior pair and has no membranous connection as suggested by Hamilton (1931).

The Prothorax

The prothorax is an independant segment having membranous connections anteriorly with the head and posteriorly with the mesothorax. The

[J. Anim. Morph. Physiol., Vol. 8, No. 2, pp. 81-92, 1961]

tergum, pleura and sternum are fused together without any trace of tergopleural and sternopleural lines. Their respective limits can be drawn by imaginary tergopleural and sternopleural lines which can be shown as extensions of the respective lines from the abdomen. The first spiracle is located at the posterior part of the prothorax roofed over by the posteriorly projecting protergum.

The protergum is collar-shaped in dorsal view and posteriorly it covers the anterior region of the mesonotum. There are three longitudinal carinae on the tergum extending from the anterior to the posterior region. Of the three carinae, one is median and the remaining two occupy lateral positions. The lateral carinae are convergingly curved posteriorly. The protergum possesses no sutures unlike that of meso and metatergum. The anterior and posterior margins of the protergum develop thin ridges for the attachment of the neck membrane and intersegmental membrane respectively.

The propleura and sternum are fused together to form the propectus. It occupies the lateroventral and ventral regions of the prothorax. The pleuron is reduced and is attached anteriorly to the inner side of the tergum. The tergum is extending over the pleuron for a little distance. The pleural suture is hardly discernible, but the pleuron is reinforced by pleural ridge extending upward from the pleural articular process. So the pleuron can be divided into an anterior episternum and a posterior epimeron. The episternum bears anteriorly the cervical apodemes. Laterally, it extends as a small sclerite, the precoxale, to join the sternum. Posterior to the leg the epimeron is continued to the sternum by a small sclerite, the postcoxale. The leg is attached to the pleuron in the middle by the pleural articular process. Besides this, the trochantin articulates with the leg at its anteroventral side. The trochantin arises from the pleural ridge region, or at the place where the two supracoxal arches are united. The trochantin is broad at the base and gradually tapers to the tip. It curves round to join the coxal margin. From the pleural ridge arises the pleural apophyses which join with the corresponding sternal apophyses to form the furca. The prothoracic furca is very small corresponding to the small size of the propectus.

The prosternum is a narrow sclerite extending ventrally between the propleura. In the middle it is divided by an incomplete transverse suture, the sternacostal suture, which bears internally the sternacosta. This

suture divides the prosternum into an anterior basisternum and a posterior sternellum. The sternal apophyses arise from the lateral side of the sternacosta and extend dorsolaterally to join with the pleural apophyses of the corresponding sides to form the furca.

The Mesothorax

The mesothorax is the largest segment and occupies nearly two-thirds area of the thorax. There are distinct tergopleural sutures separating the tergum from the pleura. The mesopleura and the mesosternum are fused to form the mesopectus.

The mesonotum is a large, arched plate pointed posteriorly. It has three mediolongitudinal carinae occupying the entire dorsal surface. The disposition of the mesothoracic carinae is almost the same as that of the prothoracic carinae. The mesonotum is divided into two distinct regions, an anterior prescutum and a posterior scutoscutellum, by a transverse suture, the prescutal suture. Towards the anterior side, at the submarginal area, there is another suture called the antecostal suture. It separates a narrow marginal area, the acrotergite, and bears internally a submarginal antecosta. The antecosta bears the first phragma or prephragma. The prephragma hangs downwards freely in the thorax and is notched in the middle.

The scutoscutellar suture is absent in the mesonotum, with the result that the scutal and the scutellar areas are merged together. Posteriorly, the notum bears a reversed notal or pseudoscutellar suture. This suture divides the scutellum into a middle raised and two lateral areas. The postnotum or postscutellum of Malouf (1932) lies posterior to the scutellum and is V-shaped. It extends at the posterolateral margins of the scutellum. The axillary cord of tegmen is attached to the postnotum. In the middle, the postnotum is intimately associated with the meso-scutoscutellum and is diverged from it marginally. The postnotum bears posteriorly the unpaired postphragma. The postphragma is membranous and is deeply notched in the middle. It hangs down freely in the body cavity and occupies an intersegmental position between the mesothorax and the metathorax.

The pleural suture arises from the pleural articular process and extends upto the wing process. In the middle it is curved anteriorly. It divides the pleuron into an anterior episternum and a posterior epimeron. The former is comparatively larger than the latter. The episternum passes

to the sternum by a large precoxale. The second thoracic spiracle is situated at the lateral side of the mesothorax at the junction between the tergum and epimeron. The epimeron is joined to the sternum by a narrow postcoxale. The pleural suture internally develops the pleural ridge, which in the middle extends to form the pleural apophysis. The apophyses from the sternum join with the pleural apophyses to form the furca. At the dorsal side the episternum bears a small crescent-shaped

Fig. 1 Dorsal view of thorax.
Fig. 2 Ventral view of thorax.
Fig. 3 A. Lateral view of thorax.
B. Lateral view of metapleuron showing the internal ridge and the c nical apodeme of the trochanter (outline is drawn in dots).
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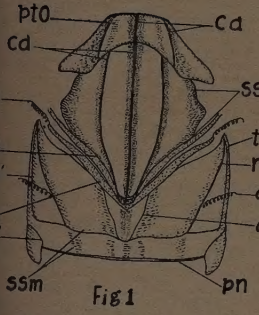
a, a' a''—sutures of metanotum; IA, IIA—first and second anals; IIA1, IIA2, IIA3—first, second and third branch of second anal; ac—axillary cord; ac2—antecosta; acr—acrotergite; ap—apodeme from the trochanter to the body cavity; ap1, ap2—apophyses from the pleural suture to the pleural ridge. ar—arolium; 3ax—third axillary; axa—auxilia; b—suture on the metapluron; ba—basalare sclerite; bc—basicoxite; bcs—basicostal suture; br1, br2—branches of the pleural ridge; bs—basisternum; c—suture on the metasternum; C—costa; ca—carinae; cl—claw; cp—cervical apodeme; Cu1, Cu2—first and second branches of cubitus; Cula, Culb—first and second branches of first cubitus; cx—coxa; d, d'—sutures on the mesosternum; e—suture above the branch (brl) of pleural ridge towards the posterior side: em—epimeron; es—episternum: f1 + 2—fused first and second axillaries; fe—femur; hp—humeral plate; imb—intersegmental membrane between the pro and mesothorax; imm—intersegmental suture between the meso and metapleuron; is—intersternite; lt—last tarsomere; M—media; M1, M2, M3—first, second and third branches of media; mp—median plate; mps—median plates; mts—metasternum; pc—precoxale; pes—presternal suture; ph—first phragma; pl—concavity of coxa for the articulation of pleural process; plr—pleural ridge; pls—pleural suture; pn—postnotum; pnm—presternum: prs—prescutum; pss—prescutal suture; pta—pretarsus; ptc—postcoxale; pto—pronotum; R—radius; R1—radial one; Rs—radial sector; rvs—reversed notal suture; sa—subalare; Sc—subcosta; Sc1, Sc2—first and second branches of subcosta; Sc + R—subcosta plus radius; scs—sternacostal suture; sl—sternellum; sp—spine on the hind coxa; sp2—second thoracic spiracle; spr—spur; sr—sclerotic rim at the margin of conical apodeme; ssm—scutoscutellum; ta—tarsus; tg—tegula; ti—tibia; tn—trochantin; tha—cavity on the coxa for the articulation of trochantin; tps—tergopleural suture; tr—trochanter; uf—unguifer; utr—unguitractor.



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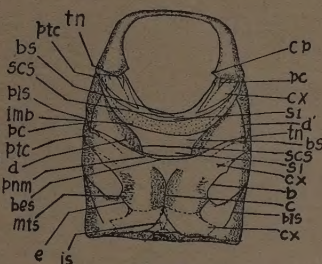

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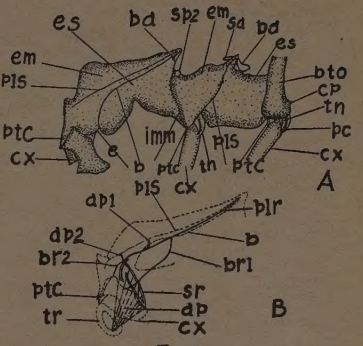

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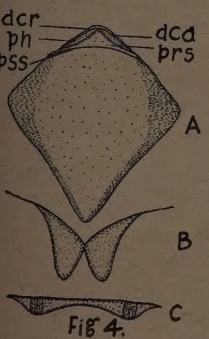

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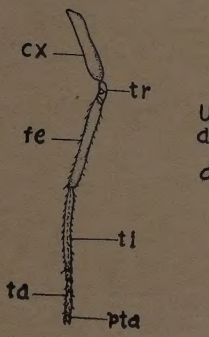

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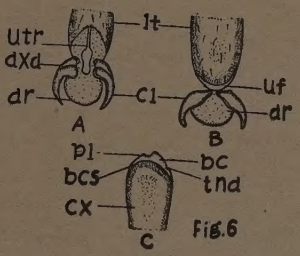

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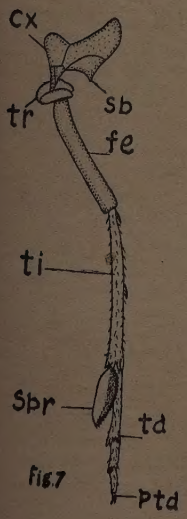

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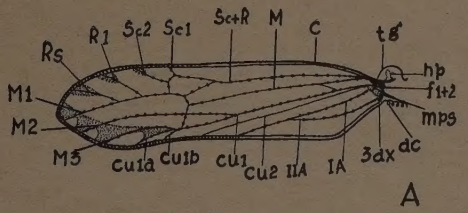

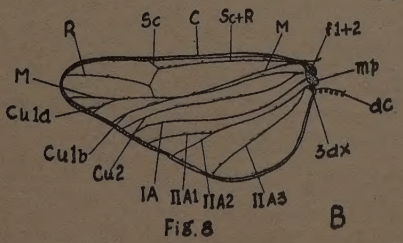

Fig.8

sclerite, the basalare. Similarly the epimeron bears another small sclerite, the subalare. The trochantin is similar to that of the prothorax in origin, articulation and shape, and has proportionally enlarged with the increase in size of the mesothorax.

The eusternum of the mesothorax is limited posteriorly by the intersegmental groove. The sternacostal suture divides the sternum into an anterior large basisternum and a posterior small sternellum. The sternellum is rectangular in outline. The sternacostal suture internally bears the sternacosta, the lateral ends of which carries the sternal apophyses. There is a pair of sutures (Fig. 2, d) on the basisternum extending from the lateral margins of the sternacostal suture to the anterior margin. Another pair (d') is present on the sternum at its lateral sides extending longitudinally from the posterior to the anterior margin.

The Metathorax

Distinct tergopleural lines separate the metanotum from the metapleura. Similar to the propectus and the mesopectus, the pleura and sternum fuse to form a metapectus. The metapectus is highly modified as the leaping mechanism is connected to it. Curiously enough, the metathorax is devoid of a furca.

The metanotum is fairly developed and lies between the mesonotum and the first abdominal terga. It is broader than long. Laterally, it is fused with the metapectus along the tergopleural lines. Similar to the mesonotum, the scutoscutellar suture is absent in the metanotum and has a V-shaped pseudoscutellar suture. The axillary cord of the hind wing is attached to the latter suture. The scutoscutellum has a conical suture (Fig. 1, a) internal to the pseudoscutellar suture bounding an area of the scutoscutellum. Besides, there is a pair of sutures (a', a'') on either side of the pseudoscutellar suture. The a' runs parallel to the tergopleural suture and at the posterior side turns at right angles to join with the pseudoscutellar suture. The other suture (a'') is small, curved and is situated at the posterolateral angle of the metanotum. The postnotum is reduced and is represented by a narrow transverse plate. It internally carries the unpaired phragma which hangs freely into the body cavity. The phragma is strengthened at the lateral sides by two longitudinal sclerotisations.

Metapleuron is well-developed and extends between the mesopleuron and the first abdominal segment. The pleural suture extends from the

wing process and divides the pleuron into an anterior large episternum and a posterior small epimeron. Ventrally, a suture (Fig. 3, B, b) runs parallel to it. It curves round at the posterior region and proceeds to join the intersegmental suture between the mesopleuron and the metapleuron. It takes an irregular course. The episternum passes to the sternum by a large precoxale. At the dorsoanterior margin of the episternum is discernible a small sclerite, the basalare. The postcoxale is much reduced and is represented by a narrow region. The subalare is reduced and is not marked in the epimeron. Unlike that of the coxae of fore and midlegs, the hind coxa is firmly united with the pleuron. Since the coxa has lost its movement, no distinct trochantin is present in the metathorax. The pleural suture internally develops the pleural ridge. The pleural ridge and its course deserves special attention as it has undergone great modification. The pleural suture extends from the wing base and stops a little anterior to the coxa. However, the pleural ridge is joined to the coxal base. It is in close association with the pleural suture at the wing base but is separated posteriorly from it by a space. Posteriorly the free part of the ridge is supported by two apodemes (Fig. 3, B, ap1, ap2) from the pleural suture. From the middle the pleural ridge gives a ventral branch (br 1), marked externally by short suture (Fig. 2, e) towards the coxal base, which in its turn bifurcates distally and joins the coxal base. The pleural ridge gives another branch (Fig. 2, B, br2) to the posterolateral corner of the metathorax. All these ridges form a frame work to support the pleuron to which the coxa is immovably articulated.

The metasternum is comparatively well developed. The sternacostal suture is indistinct and the sternacosta is extending longitudinally as a long thin ridge, marked externally by a suture (Fig. 2, c). Hence it is difficult to say the exact boundary between the basisternum and the sternellum. The sternal apophysis is reduced. The anterior part of the sternum has a submarginal suture, the presternal suture, which cuts a narrow marginal presternum. Posterior to the sternum an intersternite is developed.

The Legs

The leg is divisible into coxa, trochanter, femur, tibia, tarsus and pretarsus. The coxo-trochanteral, the trochantero-femoral and the femorotibial joints are dicondylic, while the rest are monocondylic. All the dicondylic articulations are horizontal except the trochantero-femoral which is transverse. The fore and midlegs are similar in structure and shape,

while the hind legs differ greatly from the rest as they have undergone profound modifications for the leaping purpose.

The coxae of the fore and midlegs are elongated with a slightly broad base. Proximally, the coxa possesses a distinct rim with a small concavity at its apex for the articulation of pleural articular process. Anterior to it there is another concavity on the coxal rim to which is articulated the apex of the trochantin. Thus the coxa has a pleural as well as a trochantinal articulation. The coxal corium attaches the base of the coxa with the coxal socket. The basal part of the coxa is encircled by a submarginal basicostal suture. The suture internally gives a ridge, the basicosta, and sets off a marginal flange, the basicoxite. The basicosta strengthens the base of the coxa. The distal rim of the coxa possesses a pair of articular knobs to form a dicondylic articulation to the trochanter.

The trochanter is the smallest segment of the leg. Its walls are highly sclerotised and its lumen is reduced due to the formation of distinct marginal inflections from the wall. The dorsal surface is short and almost concave while the ventral surface is longer and convex. From the trochanteral base arises a pair of thin long apodemes, one on either side, from the points of articulation of the trochanter with the coxa. They lie inside the coxa. They are attached to the trochanteral base by membrane and serve for muscle attachments. As already mentioned the trochanter has a dicondylic articulation with the femur. The trochantero-femoral joint is so fixed that usually the femur moves along with the trochanter.

The femur is the longest segment of the leg. It bears three longitudinal rows of spines, one on dorsal and two on ventral side. No apodemes are attached to the base of the femur.

The tibia is almost of the same size as that of the femur. It bears five longitudinal rows of spines. Three of them are distributed similar to the femur, while the rest two occupy lateral positions. Towards the distal end the spines are crowded together. A pair of apodemes take their origin from the basal margin of the tibia. They extend in the femur and serve for muscle attachments. The distal part of tibia has a concavity and the first segment of tarsus fits into it so as to give a monocondylic articulation.

The tarsus is considerably shorter than the tibia and is subdivided into three tarsomeres. Of the three, the basal two are almost equal in length while the distal one is approximately equal to the combined length of the first and second. The tarsus is covered by scattered spines. The

distal end of the first tarsomere bears a crescent-shaped concavity in which the second tarsomere fits. The second tarsomere is joined to the third by a similar articulation. The basal rim of tarsus bears a pair of apodemes at its lateral sides. These apodemes extend to the tibia and the tarsal muscles are attached to it.

The pretarsus (Fig. 6, A & B) is attached to the third tarsomere distally. It consists of a pair of lateral claws (cl) and a median lobe, the arolium (ar). Each claw is articulated by a membrane to the dorsal side of the unguifer (uf), a median process of the distal end of the last tarsomere. The claws are hollow and curved, tapering distally. There are two small lateral plates beneath the claws, the auxiliae (axa). The arolium is also hollow with sclerotised wall and is in direct continuation with the median part of the third tarsomere (lt). On the ventral surface of the pretarsus is a median plate, the unguitractor (ut).

The hind legs are remarkable for their large size in comparison to the fore and midlegs. The coxa of the hind leg is especially modified. As already mentioned, it is firmly articulated with the pleuron and is much enlarged. Similar to the other legs there is a basicostal suture and the basicosta. Besides, the coxa is strengthened by a number of ridges (Fig. 7, cx). At the outer side it bears a large spine. The coxo-trochanteral articulation is movable. The trochanter is small and is strengthened by inflections from the wall. From the anterior margin towards the inner side, the trochanter gives an apodeme (Fig. 3, B, ap). It is extending into the body through the hollow coxa. The inner end of this apodeme becomes circular, rimmed by strongly sclerotised border (sr.). The apodeme is conical in shape. Muscles from the thorax are attached to the circular surface of this apodeme. The trochantero-femoral articulation is fixed and the conical apodeme moves along with the movement of the femur. The apodeme with its attached muscles facilitates to take longer leaps. However, Quadri and Aziz (1950) report in *Pyrilla perpusilla* that a conical apodeme of this type arises from the base of the coxa.

The femur is devoid of spines. The tibia has five rows of small spines. At the posterior side they are scattered. Besides, there is a large basal and a middle spine and a cluster of five spines at the hind region. The hind tibia bears a foliaceous spur or calcar of unknown function. At the posterior half it is provided with scattered minute spines, and the hind margin bears a number of teeth.

Of the tarsomeres the basal one is the longest instead of the distal one as in the fore and midlegs. At the hind end of the first tarsomere there is a cluster of seven enlarged spines, of which five are in one cluster and the rest two in the other. The second and third tarsomeres are almost equal in length. The former bears at its hind end a cluster of four enlarged spines. The pretarsus is similar in structure to that of other legs.

The Wings

The wings are well developed and extend beyond the abdomen. The forewings or tegmina are of slightly harder consistency than the hind wings. The former are narrower and longer than the latter. It is ochraceous with fuscous markings distally (Fig. 8, A). The hind wings are ochraceous.

The wing venation is comparatively well developed. Muir (1923) observed for the first time that the 'Y' vein of Fulgoroidea was formed by the union of first and second anal veins. In the tegmen the costa is marginal and unbranched. Subcosta is two-branched, subcosta 1. and subcosta 2. Basally the subcosta is united with the costa. The radius coalesces with the subcosta for about half of its length and bifurcates distally, the branches represent the radial 1. and radial sector. Media is three branched, the media 1., media 2. and media 3. According to Metcalf (1913) in Fulgoroidea the media 3. is formed by the union of the media 3. + 4. during the course of its development. Basally the media apposes the fused subcosta and radius. Cubitus is two-branched, cubitus 1. and cubitus 2. Basally the two branches unite to form a common stalk. The cubitus 1. in its turn branches into Cula. and Culb. The anal vein is two-branched, the first and second anals. Distally, they fuse to form the characteristic 'Y' vein of Fulgoroidea. All the veins, except the cubitus 2., of the tegmen is provided with macrotrichiae.

The hind wing is striking for its enlarged anal region, reduced number of veins of subcosta and media and the complete absence of macrotrichiae on the veins. The costa runs along the anterior border of the wing and unites basally with the subcosta. The subcosta runs parallel to the former and is unbranched. The radius as well as the media are unbranched. Basally, the subcosta, radius and media fuse to form a common stalk. Media coalesces with the anterior branch of cubitus 1., (Cula), for a short distance and diverges from it at the distal part. The

branching of the cubitus is similar to that of the tegmen. The anal vein forks into two main branches, IA. and IIA. The former is unbranched while the latter is three-branched (IIA1, IIA2, IIA3) covering the anal area.

The membranous wing base contains a number of articular sclerites, the pteralia. The axillary membrane is thickened and corrugated at its posterior margin to form the axillary cord. The pteralia of the tegmen consist of the following :

Humeral plate : It is a small sclerotised plate at the anterior margin of the wing base having close connection with the costal vein. The tegula is considerably enlarged and overlaps the humeral plate.

First and second axillaries : They are fused to form a small elongated sclerite, situated between the median and humeral plates. It is curved, with its base touching the median plate.

Median plates : They consist of a large basal and a small distal plate situated between the third axillary and the fused first and second ones.

The third axillary : It is a small triangular sclerite situated at the posterior margin of the tegmen, posterior to the median plate. Mesally, it touches the median plate.

The relative position of the sclerites in the axillary membrane of hind wing is the same as that of the tegmen, though they differ in their shape and size. In the hind wing, the humeral plate is reduced and the median plate is represented by a single sclerite.

Summary

The external morphology of the thorax of *Peregrinus maidis* is described in detail.

The metathorax is devoid of furca. The plural suture stops a little above the coxa while the ridge extends to the coxa. Posteriorly, the ridge is departed from the suture by a space and is supported by two apodemes from the latter. The ridge in its course branches to strengthen the pleuron.

The hind coxa is enlarged and is firmly joined to the pleuron. The trochanter develops a conical apodeme which is extending into the body cavity. The hind tibia bears a foliaceous spur with teeth on the hind margin.

The first and second axillaries of the tegmen as well as the hind wing are fused together. In the hind wing the median plates are fused to form a single sclerite. In the tegmen a characteristic 'Y' vein is formed by the union of the anals, and all the veins, except the cubitus 2., is provided with macrotrichiae.

Acknowledgements

We are thankful to Sri. Bhim Sen, Principal, Government College, Ajmer, for giving all the facilities and to Mr. D. C. Pant, for his assistance in the preparation of the diagrams.

References

1. Akbar, S. S., 1957 The morphology and life history of *Leptocorisa varicornis* Fabr. (Coreidae, Hemiptera)—A pest of paddy crop in India. Alig. Musl. Univ. Publ. (Zool. Ser.) Ind. Ins. Typ. Pp. 53.
2. Crampton, G. C., 1909 A contribution to the comparative morphology of the thoracic sclerites of insects. *Proc. Acad. Nat. Sci.* Philadelphia, 3-54.
3. Hamilton, M. A., 1931 The morphology of the water scorpion, *Nepa cinera* (Heteroptera, Rhynchota). *Proc. Zool. Soc.* London, 1067-1136.
4. Malouf, N. S. R., 1932 The skeletal-motor mechanism of the thorax of stink bug '*Nezara viridula* L.' *Bull. Soc. Ent. Egypte*, Cairo, **16**, 161-203.
5. Martin, J. G., 1916 Thorax and cervical sclerites of insects. *Ann. Ent. Soc. Amer.*, **9**: 35-83.
6. Metcalf, Z. P., 1913 The wing venation of the Fulgoroidea. *Ann. Ent. Soc. Amer.*, **6**: 341-352.
7. Muir, F., 1923 On the classification of the Fulgoroidea. *Proc. Haw. Ent. Soc.*, **5** (2), 215-218.
8. Myers, J. G., 1928 Morphology of Cicadidae. *Proc. Zool. Soc.* London, No. XXV, 365-472.
9. Qadri, M. A. H, and Aziz, A, 1950 Biology, life history, and external and internal anatomy of *Pyrilla perpusilla* Walker. Alig. Musl. Univ. Publ. (Zool. Ser.) Ind. Ins. Typ Pp. 33.
10. Snodgrass, R. E, 1909 The thorax of insects and the articulation of the wings. *Proc. U. S. Nat. Mus.* **36**, 117-136.
11. ., 1935 Principles of Insect Morphology. McGraw-Hill Book Co., N. Y., Pp. 667.

[Received for publication on 18th February, 1961.]

STUDIES ON THE EXTERNAL MORPHOLOGY OF *Peregrinus maidis* (Ashmead)

(Homoptera, Fulgoroidea, Araeopidae = Delphacidae)

Part III, Abdomen

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THIS paper is the last in the series of studies of the external morphology of *Peregrinus maidis* and deals with the abdomen. The abdomen is the largest part of the body and is elongate, subcylindrical, tapering posteriorly. It consists of ten well defined segments, with a small posterior eleventh one fused to the tenth segment. The abdominal segments regularly overlap posteriorly, except where they are fused together, and the first eight segments bear spiracles. They can be grouped into pregenital, genital and postgenital segments. The ninth segment in the male and the eighth and the ninth in the female represent the genital segments.

Material and Method

The abdomen was made transparent by the action of cold concentrated potassium hydroxide solution for six to eight hours at laboratory temperature and studied under stereoscopic binocular microscope. The transparent male genitalia were dehydrated in a mixture of carbolxylol and mounts of different parts were made as illustrated by Giffard (1921). The various parts of the female genitalia were mounted in the balsam in the usual way.

Description of Pregenital Segments

(a) Male. (Figs. 1, 2) The pregenital region consists of the first eight segments. A typical segment consists of four chitinised plates, a dorsal arched tergum, two small lateral conical plates, the laterotergites and a ventral pleurosternite. The tergum has a mediolongitudinal sclerotised area. Its antecostal suture is marginal and is hardly discernible. The antecosta of the tergum is thin and the acrotergite is a scarcely perceptible marginal rim. The laterotergites belong to the tergal region and bear the spiracles. Snodgrass (1935) has clearly demonstrated that the spiracles invariably belong to the tergum. However, according to Myers

[J. Anim. Morph. Physiol., Vol. 8, No. 2, pp 93-99, 1961]

(1928) and Qadri and Aziz (1950) they belong to the sternal and pleural parts in Cicadidae and *Pyrilla perpusilla* (Fulgoroidea) respectively. The spiracles have the internal closing apparatus. The pleurosternite is formed by the union of the pleura with the sternum. The disposition of the antecosta, the antecostal suture and the acrosternite are similar to that of the tergum.

The first segment is fused with the metathorax and its acrotergite with its antecosta have merged anteriorly to form the postnotum and the phragma of metanotum respectively. The first and the second segments are reduced and are fused together. Their terga are sclerotised transverse plates with the spiracles included in it. There is no laterotergite in these two segments. In the second tergum the antecosta is obliterated. It bears a faint transverse suture (a) at its anterior region. The second spiracle is surrounded by a considerably developed peritreme. The first and the second pleurosternites are folded between the metasternum and the third pleurosternite. The antecostal suture and the antecosta are not discernible. The third segment is a typical one. Its antecosta is well developed and the pleurosternite bears hairs at the posterior border. The

Fig. 1 Male abdomen (dorsal view).
Fig. 2 Male abdomen (ventral view).
Fig. 3 Female abdomen
A. Dorsal view of the first and the second segments.
B. Ventral view.
Fig. 4 Male genitalia and the last three segments (ventral view).
Fig. 5 A. Single genital style (lateral view).
B. Aedeagus periandrium showing the basal sclerite.
Fig. 6 Femal genitalia dissected.
Fig. 7 Tenth and eleventh segments.
A. Male (in situ).
B. Female (eleventh segment partly extended).

a—transverse suture on the second tergum; abs—aedeagus basal strut; ae—anal emargination; ap—aedeagus periandrium as—anal style; bpb—basal plate bridge; bpp—basal plate prolongation; cd—position of condyle; gs—genital style; ig—inner margin of genital style; ir—inner ramus of the first valvula; is—intersternite; ism—intersegmental membrane; lt—laterotergite; mls—mediolongitudinal sclerotised area of sternum; mlt—mediolongitudinal sclerotised area of tergum; nt—ninth tergum; og—outer margin of the genital style; or—outer ramus of the first valvula; p—chitinised process on the tenth segment; pm—postnotum of the metanotum; py—pygofer; sgp—subgentital plate; sp—spiracle; st—strut directed anteriorly from the eleventh segment; tap—tooth of aedeagus periandrium; tm—tergum; ts—transverse sclerite connecting the chitinous processes of the tenth segment; 1vf—first valvifer; 2vf—second valvifer; 1vl—first valvula; 2vl—second valvula; 3vl—third valvula; wg—wing of the bridge; 1-11—one to eleven segments: 10 + 11 fused tenth and eleventh segments.

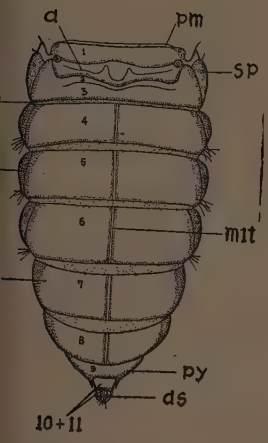
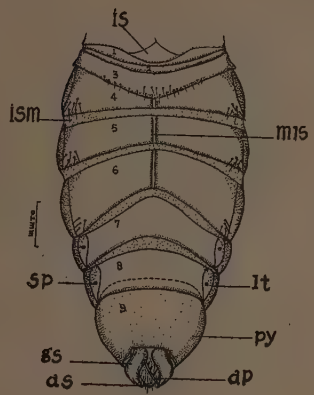


Fig.1



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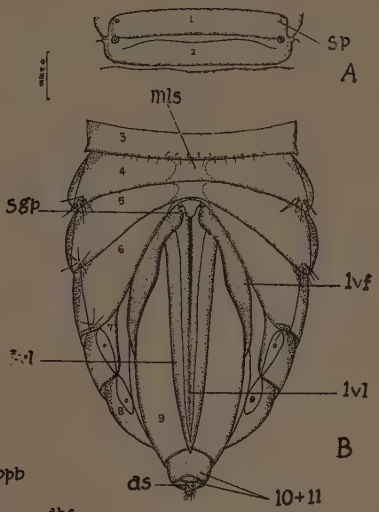

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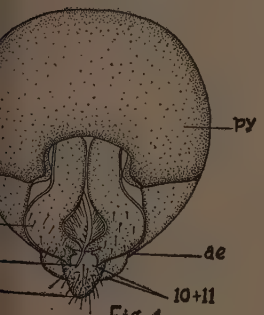

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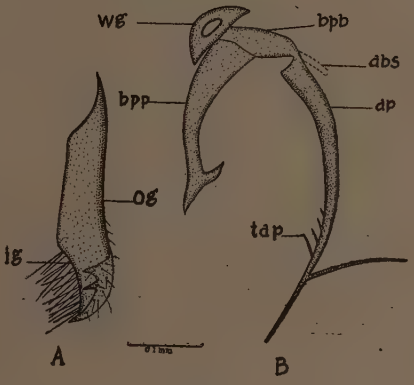

Fig.5

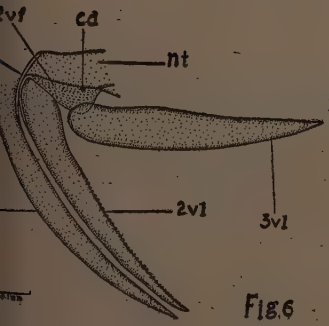

Fig.6

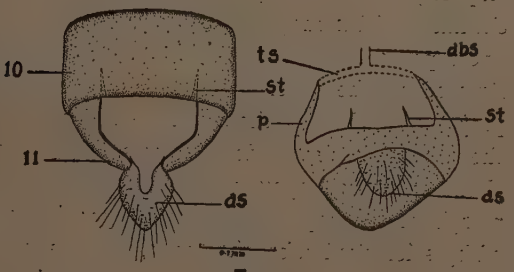

Fig.7

segments progressively enlarge from the third to the sixth and then decrease in size posteriorly. The pleurosternites of the fourth, the fifth and the sixth segments have mediolongitudinal sclerotised areas corresponding to the tegra. They bear clusters of long hairs on the lateral sides, their number varies from four to six.

(b) Female. (Fig. 3) The structure of the pregenital segments in female is essentially similar to that of the male save for the following differences. The abdomen in the female is larger than that of the male and the pregenital segments consist of the first seven segments. The second tergum bears an antecostal suture, an antecosta and an acrotergite unlike that of the male.. The seventh pleurosternite is practically reduced and bears a triangular subgenital plate.

Genital Segments

(a) Male. (Figs. 4, 5) In the following description of the male genital segment and the genitalia, the terminology of Giffard (1921), Pruthi (1925) and Muir (1926) has been employed. The tergal, pleural and sternal plates of the ninth segment are confluent in a continuously sclerotised ring, the pygofer, forming an armature around the genitalia. The pygofer is black and is quite conspicuous with an entire ventral margin. Its posterior margin is deeply emarginated, the anal emargination, surrounding the tenth and the eleventh segments. The corners of the anal emargination, the anal angles, are acute. The opening of pygofer is longer than broad. The intersegmental membrane between the ninth and the tenth segments is known as the diaphragm. The latter divides the pygofer into an inner and an outer chamber, and its dorsal margin is 'V' shaped.

A pair of genital styles project through the ventral margin of the diaphragm. They are parallel to each other. Each genital style is simple, chitinised, sickle-shaped sclerite bearing hairs distally. The hairs are of two types, small hairs scattered on the outer surface and the long hairs situated at the inner margin. Dorsally, the genital style bears three projections, of which the distal one is the largest. Behind the diaphragm the genital style suddenly tapers. The ædeagus arises from the inner chamber and its apex projects to the outer chamber over the dorsal margin of the diaphragm. It is composed of an ejaculatory duct surrounded by sclerotised tube, the aedeagus periandrium, narrow at its apex and gradually enlarging basally. The periandrium is curved and bears five

teeth directed basally. Rarely a small sixth tooth is found to occur anterior to the fifth one. Of these, the distal tooth is much longer than the remaining ones. There is a gradual increase in length from the second to the last tooth. In a few cases the distal tooth carries a small basal branch. The base of the periandrium is produced towards the tenth segment as a thin sclerotised plate, the aedeagus basal support or strut. To the base of the periandrium is attached a small sclerotised plate, the basal plate bridge, which in its turn bears proximally expanded sclerotised plates, the wings of the basal plate bridge. The bridge is joined to the genital styles by a distally bifurcated sclerite, the basal plate prolongation. This enables the insect to move the genital styles along with the aedeagus.

(b) Female. (Fig. 3B, 6.) The pleurosternite of the eighth segment is practically reduced. Its tergum is similar to the typical segment. The pleurosternite of the ninth segment is obliterated and the tergum extends ventrolaterally in the middle of the abdomen upto the posterior margin of the sixth pleurosternite. There is a mediolongitudinal groove at the ventral side of the tergum hidden by the ovipositor. Dorsally the tergum is restricted to a narrow lip.

The eighth and the ninth segments bear ventrally a well developed ovipositor. It is long extending over a little distance to the tenth segment. The ovipositor is composed of a basal apparatus, a shaft and a pair of accessory lobes. The basal apparatus consists of two pairs of plates, the first and the second valvifers, which support the ovipositor shaft. The first valvifers are elongated plates implanted in the membranous ventrolateral parts of the eighth segment. Posteriorly, they are extending about half the length of the ninth tergum and are articulated to the latter. The second valvifers are small plates concealed within the projecting lower parts of the ninth tergum. Each second valvifer is articulated at a point near the posterior side of its dorsal margin with a condyle (cd) on the ventral margin of the ninth tergum. The shaft is composed of a pair of closely appressed elongate plates, the first valvulae, over a single grooved plate, the second valvulae. They are connected each other by ridge and groove method forming a canal through which the eggs pass out. The first valvulae are extending ventrolaterally over the second and it is not possible to observe the latter in situ. The base of each valvula is produced into two rami, the outer of which is attached to the first valvifer, while the inner is fused to the ninth tergum. The second valvulae are fused together

to form a median grooved plate as already mentioned. It is highly chitinised and its dorsal surface is serrated for the distal two-thirds. The serrated nature enables the insect to puncture the plant tissues on which it lays eggs. The accessory lobes or the third valvulae are a pair of sclerotised flattened blade-like plates articulated anteriorly to the second valvifer towards the middle just ventral to the condyle. They ensheath the ovipositor, when at rest, between their concave inner surfaces.

Hassan (1948) made the first attempt to study the external genitalia of the female Araeopids while pointing out their significance in taxonomy. *He has confused their structures* and gave only a brief account. He mistook the combined first and second valvulae described in the present paper for the first, and the third for the second.

Postgenital Segments

(a) Male. (Fig. 7A) The tenth and the eleventh segments are fused to form a single structure. The tenth segment gives two lateral chitinised projections (p) directed anteriorly. They are connected with each other by a transverse thin sclerite (ts). The basal strut of aedeagus is joined to it at its middle. The tenth segment in Araeopids generally bears a pair of appendages, the anal processes, which are absent in *Peregrinus maidis*. The eleventh segment is telescoped into the tenth segment. It bears at its lateral sides a pair of struts (st) directed anteriorly and projecting into the tenth segment. Posteriorly, it gives an appendage, the anal style, bearing scattered hairs. The anus opens at the terminal region of the eleventh segment at the base of the anal style.

(b) Female. (Fig. 7B) The tenth segment is considerably large in females. It is fused with the ninth segment and there is no strut from the genital organs to the tenth segment as in the male. As regards other features, the structure of the postgenital segments is the same as that of the male.

Summary

This is the first detailed study of the morphology of the abdomen of an Araeopid, *Peregrinus maidis*.

1. The abdomen is composed of eleven segments. A typical segment consists of a tergum, two laterotergites bearing spiracles and a pleurosternite formed by the union of pleura with the sternum.

2. The opening of pygofer is longer than broad. The genital styles are sickle-shaped. The aedeagus bears five or six teeth directed posteriorly.

3. The ovipositor is composed of two valvifers each bearing three valvulae. The first valvulae are lateral to the second and the outer rami of the former are joined to the ninth tergum. The second valvulae are fused together to form a single piece.

4. The tenth and the eleventh segments are fused together. The anal processes are absent. Thc anus opens on the eleventh segment. The aedeagus is connected to the tenth segment by a basal strut.

Acknowledgments

I am greatly indebted to Dr. P. N. Mathur, Head of the post graduate Department of Zoology, Government College, Ajmer, under whose supervision this work has been carried out. I am also grateful to Professor Bhim Sen, Principal, Government College, Ajmer, for giving me all the research facilities and to Mr. Oommen Jacob for his assistance in the preparation of the diagrams.

References

Giffard, W. M., 1921 The systematic value of the male genitalia of Delphacidae (Homoptera). *Ann. Ent. Soc. Amer.*, **14** : 135-140, Columbus.

Hassan, A. I., 1948 The significance of genitalia in generic determination of Araeopidae (Delphacidae). *Bull. De La Soc. FouadLer D'Entomologie*, **32** : 83-93.

Muir, F., 1926 The morphology of the aedeagus in Delphacidae (Homoptera). *Trans. Ent. Soc. Lond.*, **74** : 377-380.

Myers, J. G., 1928 Morphology of Cicadidae. *Proc. Zool. Soc. Lond.*, No. 25, 365-472.

Pruthi, H. S., 1925 The morphology of the male genitalia in Rhynchota. *Trans. Ent. Soc. Lond.*, 127-267.

Qadri, M.A.H, & Aziz, A., 1950 Biology, life history and external and internal anatomy of *Pyrilla perpusilla* Walker. Alig. Musl. Univ. Publ. (Zool. Ser.) Ind. Ins. Typ. pp. 33.

Snodgrass, R. E., 1935 Principles of Insect Morphology, McGraw-Hill Book Co., N.Y., Pp. 667.

[Received for publication on, 18th, February, 1961]

COMPARATIVE EFFICACY OF EGG-YOLK-BUFFER DILUENTS CONTAINING CARBON DIOXIDE FOR THE PRESERVATION OF BULL SEMEN

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Ever since the discovery of the egg-yolk-phosphate (EYP) diluent by Phillips and Lardy (1940) for preservation of bull semen, a variety of semen diluents have been used in routine artificial insemination. Salisbury *et al.* (1941) replaced phosphate buffer with citrate buffer in the diluent, which improved preservation of spermatozoa. Other efforts to improve upon the egg-yolk-buffer diluent consisted in the addition of (i) gelation (Knoop 1941), (ii) glycine (Knoop and Krauss 1944, Roy and Bishop 1954), (iii) antibacterial agents (Knodt and Salisbury 1946, Almquist *et al.* 1946, Phillips and Spitzer 1946 and others), (iv) sodium bicarbonate and glucose (Kampschmidt *et al.* 1951) and (v) CO_2 (Sharma 1957).

After the publication of the first encouraging report on the Illini Variable Temperature (I.V.T.) diluent, several other studies have confirmed the earlier findings (Jaskowski 1958, Scott and Hardenbrook 1958, Becze 1960). Sharma and Mahajan (1960) reported that the motility of buffalo spermatozoa could also be preserved in the I.V.T. diluent near optimum level for a period of 4 to 6 days. The chief ingredient in the I.V.T. diluent which renders room temperature preservation of semen possible is the CO_2 gas and as such a general use of CO_2 in bull semen diluents may result in improved sperm storage.

The object of the study reported here was to find out the relative efficacy of different buffers with CO_2 in the preservation of bull semen both at refrigerator and room temperatures.

Materials and Methods

Semen used came from five *Hariana* bulls kept under optimum conditions of nutrition. In all, 21 ejaculates were studied and split samples from each diluted at levels varying between 1 : 25 and 1 : 40 were preserved in the diluents detailed below :

(i) *Citrate buffer*

Sodium citrate dihydrate = 2.90 gm.

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	($Na_3C_6H_5O_7.2H_2O$)	
	Glass distilled water	= 100 ml.
(ii)	*Phosphate buffer*	
	Disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$)	= 2.00 gm.
	Potassium dihydrogen phosphate (KH_2PO_4)	= 0.20 gm.
	Glass distilled water	= 100 ml.
(iii)	*Glucose-bicarbonate buffer*	
	Glucose	= 4.00 gm.
	Sodium bicarbonate ($NaHCO_3$)	= 0.26 gm.
	Glass distilled water	= 100 ml.
(iv)	*Glycine buffer*	
	Glycine	= 4.00 gm.
	Glass distilled water	= 100 ml.
(v)	*I. V. T. buffer*	
	Sodium citrate dihydrate	= 20.01 gm.
	Sodium bicarbonate	= 2.01 gm.
	Potassium chloride	= 0.40 gm.
	Glucose	= 3.00 gm.
	Glass distilled water	= 1000 ml.

All the chemicals used in these investigations were chemically pure and reagent grade. The required quantities of the chemicals were accurately weighed and dissolved in requisite quantities of glass distilled water.

To these buffers sulphanilamide 3 mg. per ml. (dissolved by heating), penicillin 1000 i. u. per ml. and streptomycin 1 mg. per ml. were added. The buffers were then gassed with CO_2 for about 10 minutes and egg yolk added to give a concentration of 10%.

The diluted semen was immediately pipetted into glass ampoules of about 1 ml. capacity and sealed hermatically. Half of these ampoules were kept at room temperature (22-33°C) and the other half were transferred to a beaker containing water and stored in a refrigerator at 4-6°C.

Motility estimates were made once daily in a Blom's Comparing Chamber at 37°C over a period of seven days. A numerical value ranging from zero to five was given to each sample at the time of motility estimation. Here zero indicated no motility and five indicated the maximum motility. Half grades were frequently given to describe the interme-

diary motilities. Each full grade represented 20% motile sperm. These motility scores were therefore multiplied with 20 to get the per cent motile sperm.

Results and Discussion

The average numbers of spermatozoa surviving in the five diluents at room temperature and in refrigerator are given in Table I and are presented graphically in figures I and II,

TABLE I

Per cent motility of bull spermatozoa during storage in refrigerator and at room temperature in five different diluents

(Average of 21 ejaculates)

Age of semen (in days)	Per cent motile sperm									
	Citrate		Phosphate		Glucose-bicarbonate		Glycine		I.V.T.	
	4-6°C	22-33°C	4-6°C	22-33°C	4-6°C	22-33°C	4-6°C	22-33°C	4-6°C	22-33°C
0	72	72	72	72	72	72	72	72	72	72
1	67	52	21	14	68	48	11	23	66	52
2	59	33	4	5	66	29	1	4	63	38
3	56	22	1	3	64	20	—	—	61	33
4	54	17	—	2	62	14	—	—	57	29
5	48	12	—	2	57	9	—	—	52	24
6	41	8	—	1	51	5	—	—	48	20
7	33	6	—	1	48	2	—	—	41	10

It is evident from the foregoing results that the three diluents namely I.V.T., yolk-glucose-bicarbonate and yolk-citrate maintained a much better motility upto 7 days than yolk-phosphate or yolk-glycine diluent. The differences between the former and the latter group of diluents were highly significant. At refrigerator temperature, the differences between yolk-glucose-bicarbonate, yolk-citrate and I.V.T. diluents were not significant. However, at room temperature the I.V.T. diluent was significantly superior to yolk-glucose-bicarbonate diluent, but the differences between the I.V.T. diluent and yolk-citrate diluent at room temperature were not significant. Yolk-citrate, yolk-glucose-bicarbonate and I.V.T. diluents were significantly superior at refrigerator temperature

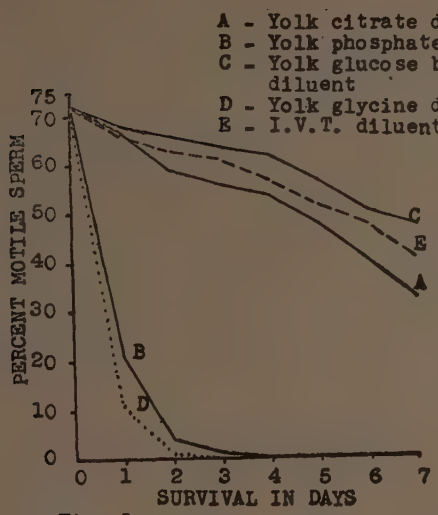

Fig. I

Per cent motile sperm during storage in refrigerator in five different buffers (Average of 21 ejaculates)

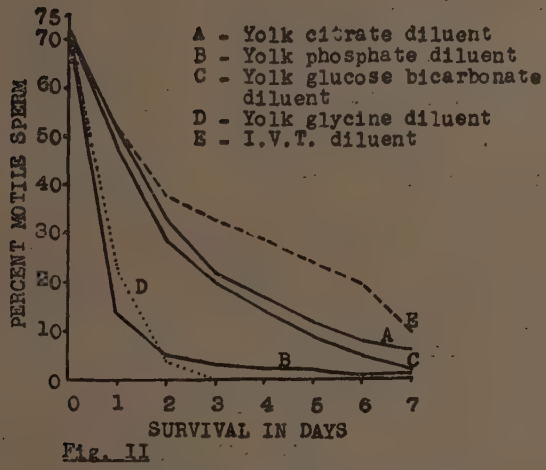

Fig. II

Per cent motile sperm during storage in five different buffers at room temperature (Average of 21 ejaculates)

than at room temperatures; however, the other two dilutors behaved similarly at both the temperatures.

At refrigerator temperature clumping together of spermatozoa in glycine preserved semen was observed. Also, in glycine diluent, there was a curdling of egg yolk both at room temperature and in the refrigerator. Statistical analysis of the data is presented in Table II.

TABLE II

Analysis of Variance

Source	*d. f.*	*M. S.*
Between treatments	4	2025.09**
Between collections	20	142.27**
Between temperature..	1	2397.09**
Interaction :		
Treatment × temperature	4	487.89**
Collection × temperature	20	34.36**
Collection × treatment	80	28.09**
Error	80	11.72
Total ..	209	—

** Significant at 1% level

There were highly significant differences between diluents, between temperatures and between ejaculates. The interactions, treatment × temperature, collection × temperature and collection × treatment were highly significant.

The semen samples were grouped according to their initial motilities which were 3, 4, or 4.5. The analysis revealed that the initial motility did not affect the preservability of semen samples under the various treatments at both temperatures in the present investigation.

Majority of the reports indicate that about 20 to 30 per cent yolk is necessary for optimum motility of bull spermatozoa during storage at 4-6°C, (Swanson 1949, Olds *et al.* 1951, Ahmed 1955, Mahajan and Sharma 1960 and others). The results of this study reveal that even 10% yolk may support motility of bull spermatozoa fairly well at 4–6°C when CO_2 is incorporated into the diluents.

The glycine diluent which has uniformly given better sperm survival (Roy and Bishop 1954, Roy 1955, Saha and Singh 1958) gave much in-

ferior results in the presence of CO_2. The curdling and flocculation in the glycine diluent may have been due to the formation of carbamino acids which are formed due to the combination of calcium in egg yolk with carbonic acid and glycine (Miller 1925). Low concentration of yolk at 10% may also have been a factor for poor sperm survival in the glycine diluent. A similar observation has been made by Foote and Bratton (1960) in connection with the glycine diluent where 20% yolk resulted in poorer preservation compared to 50% yolk.

The observations made during the course of this investigation indicate that further improvement in the methods of preserving bull semen may be possible by using modifications of the conventional citrate, glucose-bicarbonate and the I. V. T. diluents.

Summary

Preservation of 21 semen samples obtained from 5 *Hariana* bulls in citrate, phosphate, glycine, glucose-bicarbonate and I.V.T. diluents saturated with CO_2 gas and containing 10% egg yolk at dilution levels varying from 1:25 to 1 : 40 in refrigerator (4-6°C) and at room temperature (22-33°C) gave the following results :

1. The citrate, glucose-bicarbonate and the I.V.T. diluents were significantly superior in refrigerator than at room temperature.
2. The citrate, glucose-bicarbonate and the I.V.T. diluents were significantly superior to yolk phosphate and yolk glycine diluents both at room temperature and in the refrigerator.
3. The I.V.T. diluent and the citrate diluent were almost similar at room temperature but the former was significantly superior to glucose-bicarbonate diluent.
4. The initial motility of the semen samples from 3.0 to 4.5 did not affect the preservability of the spermatozoa.

Acknowledgement

The authors wish to express their gratitude to Shri P. G. Pande, M.Sc., M.R.C.V.S., Director, Indian Veterinary Research Institute, for his interest and encouragement. Sincere thanks are also extended to Dr. S. S. Prabhu, Ph. D., D.Sc., Head of Animal Genetics Division for his advice and for kindly vetting the paper and to Shri R. S. Ganeshan, Assistant Research Officer (Statistics) for advice in statistical treatment of the data.

References

Ahmad, S. I. 1955 Effect of glycine on storage of ram semen. *J. Agric. Sci.*, **46** : 164-167.

Almquist, J. O., Thorp, W.T.S. and Knodt, C. B. 1946 The effect of penicillin upon the livability, fertility and bacterial content of bull semen. *J. Animal Sci.* **5** : 400.

Becze, J. 1960 The storage of bull semen at room temperature. *Animal Breed. Abstr.* **28** : 1927.

Foote, R. H. and Bratton, R. W. 1960 Survival of bovine spermatozoa stored at 5° and 25°C in extenders containing varying levels of egg yolk, glucose, glycine, glycerol, citrate and other salts. *J. Diary Sci.* **43** : 1322-1329.

Jaskowski, L. 1958 Investigations on the preservation of bull semen. III. A diluent for the preservation of bull semen at an inconstant temperature. Preliminary data. *Animal Breed. Abstr.* **26** : 1342.

Kampschmidt, R. F., Mayer, D. T., Herman, H. A. and Dickerson, G. E.. 1951 Viability of bull spermatozoa as influenced by electrolyte concentration, buffer efficiency and added glucose in the storage media. *J. Dairy Sci.* **34** : 45-51.

Knodt, C. B. and Salisbury, G. W. 1946 The effect of sulfanilamide upon the livability and metabolism of ejaculated bovine sperm. *J. Dairy Sci.* **29** : 285-291.

Knoop, C. E. 1941 A new diluent for bovine semen. *J. Dairy Sci.* **24** : 891-892.

Knoop, C. E. and Krauss, W. E. 1944 Storage of bovine spermatozoa in diluents containing certain amino acids (Progress report). *Animal Breed. Abstr.* **16** : 123.

Mahajan, S. C. and Sharma, U. D. 1960 Effect of different proportions of egg yolk on the preservation of buffalo semen. (Under publication).

Miller, A. J. 1925 *J. Exper. Med.* **42** : 897.

Olds, D., Oliver, L. and Seath, D.M. 1951. Fertility of bull semen extended with yolk-citrate containing varying proportions of egg-yolk. J. *Dairy Sci.* **34** : 1081-1083.

Phillips, P. H. and Lardy, H. A. 1940. A yolk buffer pabulum for the preservation of bull semen. *J. Dairy Sci.* **25** : 399-404.

Phillips, P. H. and Spitzer, R. R. 1946. A synthetic pabulum for preservation of bull semen. *J. Dairy Sci.* **29** : 407-414.

Roy, A. 1955. Storage of boar and stallion spermatozoa in glycine egg-yolk medium. *Vet. Record.* **67** : 330-331.

Roy, A. and Bishop, M.W.H. 1954. Effect of glycine on the survival of bull spermatozoa *in Vitro*. *Nature* (Lond.). **174** : 746-747.

Saha, S. K. and Singh, R. B. 1958. A comparison of egg yolk-sodium citrate, egg yolk sodium citrate—glycine and egg yolk glycine as semen dilutors. *J. Dairy Sci.* 41 : 1633-1635.

Salisbury, G. W., Fuller, H. K. and Willett, E. L. 1941. Preservation of bovine spermatozoa in yolk citrate and field results from its use. *J. Dairy Sci.* **24** : 905-910.

Scott, J. V. and Hardenbrook, H. J. 1958 Studies on the use of Illini Variable Temperature semen diluent in beef cattle. *J. Amer. Vet. Med. Assoc.* **133** : 432-434.

Sharma, U. D. and Mahajan, S. C. 1960. Some observations on the preservation of buffalo semen in the Illini Variable Temperature diluent. (Under publication).

Swanson, E. W. 1949 The effect of varying proportions of egg yolk and sodium citrate buffer in bull semen dilutors upon sperm motility. *J. Dairy Sci.* **32** : 345-352.

[Received for publication on 24th March, 1961.]

EFFECT OF DIFFERENT LEVELS OF POTASSIUM CHLORIDE AND GLUCOSE ON THE PRESERVATION OF *HARIANA* BULL SEMEN AT VARIABLE TEMPERATURES

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Since the discovery of the I.V.T. diluent by VanDemark and Sharma in 1957, several workers have reported encouraging results with this diluent for the preservation of bull and buffalo semen (Jaskowski 1958 ; Scott and Hardenbrook 1958 ; Becze 1960 ; Sharma and Mahajan 1960). Senegacnik (1958) reported a better survival of bull spermatozoa at 4°C than at room temperature (20°C) in the I.V.T. diluent. These results were confirmed by Mahajan and Sharma (1961).

Lardy and Phillips (1942) reported that potassium ions were necessary for the full motility, respiration and glycolysis of the bull spermatozoa. Blackshaw (1953a, b) found that potassium reduced the adverse effect of repeated washing on bull and ram spermatozoa. He observed that the best motility of bull and ram spermatozoa was maintained with a level of 0.01 *M* of KCl though levels upto 0.04 *M* were not toxic. Sharma (1957, 1960) found a level of 0.04% of KCl to be beneficial for the survival of bull spermatozoa at room temperature. Cragle and Salisbury (1959) reported that high levels of potassium inhibited oxygen uptake and fructose utilization by bull spermatozoa.

Macleod (1941) found that human spermatozoa utilized glucose, fructose and mannose for energy purposes and the effective concentration was between 20 and 200 mg./100 ml. of diluted semen. The addition of glucose up to 116 mg. per 100 ml. of diluted bovine semen by Salisbury and VanDemark (1945) resulted in improved livability and increased production of lactic acid during incubation for one hour at 46.5°C and during storage at 5°C for 10 days. Extension of this work revealed that the addition of glucose up to 464 mg./100 ml. caused further improvement in livability with each increase in glucose level exhibiting a somewhat asymptotic approach at the highest level of glucose. Kampschmidt *et al.* (1951) showed that a diluent containing increasing proportions of glucose

[J. Anim. Morph. Physiol., Vol. 8, No. 2, pp. 107-116, 1961]

upto 3.2% was superior for preserving bull spermatozoa and Smith *et al.* (1953, 1954) confirmed these findings.

VanDemark and Sharma (1957) used 0.3% glucose in the I.V.T. diluent. Levels of glucose upto 1.0% were tested and there was an indication that further improvement may be possible by increasing glucose concentration in the diluent (Sharma 1957).

The object of the present investigation was to study the effect of different levels of potassium chloride and glucose—two of the important ingredients of I.V.T. diluent, on the preservation of *Hariana* bull semen at variable temperatures, covering a wide range.

Materials and Methods

The eight semen samples reported in experiment I came from four *Hariana* bulls. The semen was diluted in the I.V.T. diluent as described by VanDemark and Sharma (1957) at the rate of 1 : 20. The diluted semen was placed in glass ampoules of about 1 ml. capacity and sealed hermatically. The ampoules were stored at three different temperatures which were 4-6°C (in a refrigerator), 21-29°C (in an air-conditioned room) and 22-34°C (room temperature) respectively.

The 18 semen samples used in Experiment II came from four *Hariana* bulls and were diluted in the I.V.T. diluent containing 0, 0.04 and 0.08 per cent potassium chloride. Two dilution levels, namely, 1 : 20 and 1 : 200 were tried. The ampoules were stored at room temperature (16-29°C) only.

In experiment III, 18 semen samples coming from four *Hariana* bulls were diluted in the I.V.T. diluent containing 0.3, 0.6, 0.9 and 1.2% glucose. The semen was diluted at the rate of 1 : 20 and the ampoules were stored at room temperature which varied from 12-21°C. Preserved semen samples were examined for motility once daily on a warm stage at 37°C over a period of seven days. The percentages of moving spermatozoa were determined in several fields and recorded to the nearest 5%.

Results and Discussion

EXPERIMENT I—*Preservation of Hariana bull semen at three different temperature ranges in the I. V. T. diluent*

The average numbers of spermatozoa surviving in the I.V.T. diluent at three different temperatures during the first seven days of storage are given in Table I and are illustrated in Fig. I.

TABLE I

Per cent motility of bull spermatozoa during storage in I.V.T. diluent at three different temperatures

(Average of 8 ejaculates)

Storage in days	Per cent motile sperm		
	4-6°C	21-29°C	22-34°C
0	76	76	76
1	76	67	71
2	74	55	64
3	71	50	54
4	69	45	46
5	67	35	42
6	60	29	25
7	56	22	21

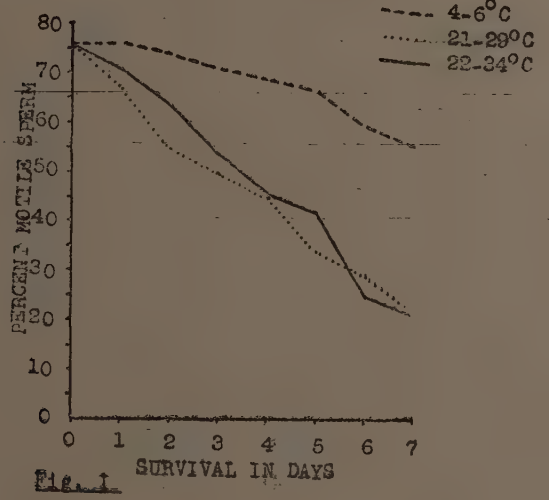

Fig. 1

Per cent motile sperm during storage in I.V.T. diluent at three different temperature ranges.
(Average of 8 ejaculates)

It is apparent from the foregoing results that bull spermatozoa preserved better in refrigerator than at room temperature (22-34°C) or in the air-conditioned room (21-29°C). The differences were highly significant. This is in agreement with the findings of Senegacnik (1958) and Mahajan and Sharma (1961). The difference between room temperature and air-conditioned room was not significant. It has been shown, therefore, that the I.V.T. diluent can be used for preserving motility of *Hariana* bull spermatozoa over a temperature range of 4-34°C; although, lower temperatures within this range are more desirable. This supports the earlier observations of Sharma (1957).

EXPERIMENT II—*Effect of different levels of KCl in the I.V.T. diluent on the preservation of Hariana bull semen at room temperature.*

The average numbers of spermatozoa surviving in different KCl concentrations at two dilution levels during the first seven days of storage are presented in Table II and graphically shown in Figs. II and III.

TABLE II

Per cent motility of bull spermatozoa during storage in I.V.T. diluent containing different levels of potassium chloride

(Average of 18 ejaculates)

Storage in days	Per cent motile sperm					
	No KCl		0.04% KCl		0.08% KCl	
	Dilution		Dilution		Dilution	
	1 : 20	1 : 200	1 : 20	1 : 200	1 : 20	1 : 200
0	76	76	76	76	76	76
1	73	70	71	73	70	73
2	64	59	58	61	59	64
3	56	47	49	48	49	55
4	52	36	46	41	40	50
5	48	30	41	34	39	43
6	44	27	36	29	35	38
7	31	21	29	24	27	28

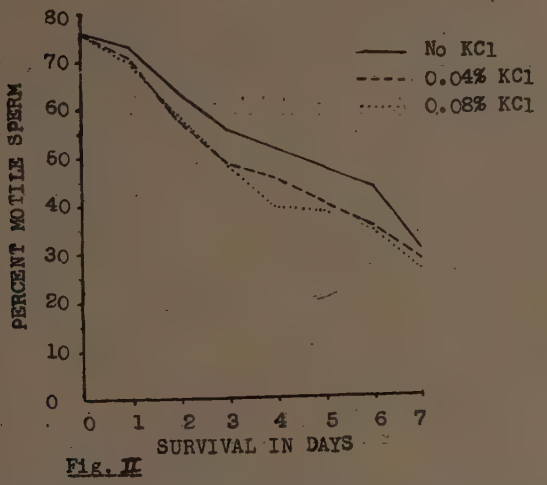

Fig. II

Per cent motile sperm during storage in I.V.T. diluent containing different levels of KCl at dilution level 1 : 20.
(Average of 18 ejaculates)

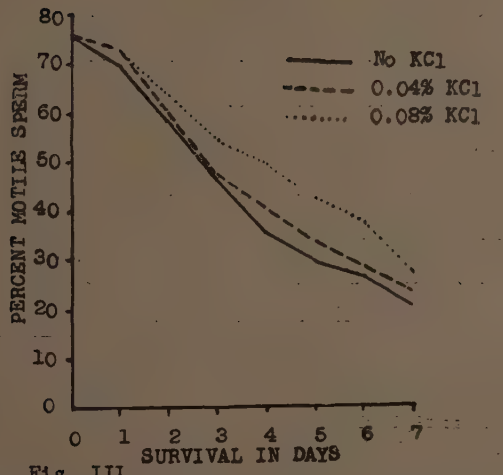

Fig. III

Per cent motile sperm during storage in I.V.T. diluent containing different levels of KCl at dilution level 1 : 200
(Average of 18 ejaculates)

The foregoing results reveal that the I.V.T. diluent containing no KCl was much superior to the diluents containing 0.04% or 0.08% KCl at the lower dilution level (1 : 20). However, at the higher dilution level (1 : 200), the I.V.T. diluent containing 0.08% KCl was significantly superior to the diluent containing no KCl. The concentration 0.08% was superior to 0.04% and the latter concentration was superior to the diluent without KCl but the differences were not statistically significant. When no KCl was present in the I.V.T. diluent, the spermatozoa preserved significantly better at dilution level 1 : 20 than at dilution level 1 : 200. However, in the presence of KCl, the differences in preservability of spermatozoa at two dilution levels were not significant. Further analysis of the pooled data revealed highly significant differences between collections ; but between dilution levels (1 : 20 and 1 : 200) and between KCl levels the differences were not significant. The analysis of variance is given in Table III.

TABLE III

Analysis of Variance

Source	*d. f.*	*M. S.*
Between treatments	2	49.31
Between collections	17	2204.68†
Between dilutions	1	238.99
Interaction:		
Treatment × dilution	2	453.39*
Collection × dilution	17	310.80†
Collection × treatment	34	87.35
Error	34	88.13
Total	107	—

† Significant at 1% level
* Significant at 5% level

White (1953) observed that potassium ions restored the motility and glycolysis of repeatedly washed bull spermatoza. He attributed the deleterious effect of washing on sperm motility to the loss of glycolytic com-

pound from the spermatozoa. Blackshaw (1953a, b) also observed the same phenomenon and found that 0.01 *M* of KCl was the optimum to prevent the harmful effect of repeated washing. Our results bear out that preservability of spermatozoa at higher dilution (1 : 200) is inferior to that at lower dilution (1 : 20). At the lower dilution, the need for KCl is not apparent; but at the higher dilution, KCl becomes increasingly useful for preserving motility of bull spermatozoa.

EXPERIMENT III—*Effect of different levels of glucose in the I.V.T. diluent on the preservation of Hariana bull semen at room temperature.*

The average numbers of spermatozoa surviving in the I.V.T. diluent containing different levels of glucose during the first seven days of storage are given in Table IV and are presented graphically in Fig. IV.

TABLE IV

Per cent motility of bull spermatozoa in I.V.T. diluent containing various levels of glucose

(Average of 18 ejaculates)

Storage in days	Per cent motile sperm			
	0.3% glucose	0.6% glucose	0.9% glucose	1.2% glucose
0	89	89	89	89
1	71	84	88	89
2	55	76	85	85
3	49	67	81	80
4	46	60	77	78
5	43	49	71	75
6	37	46	68	72
7	29	42	58	64

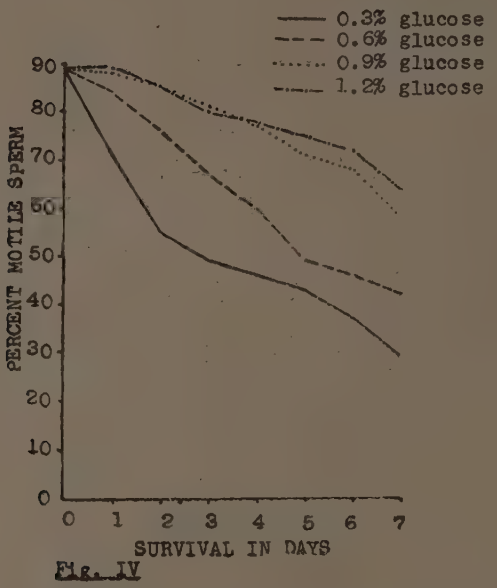

Fig. IV

Per cent motile sperm during storage in I.V.T. diluent containing various levels of glucose.
(Average of 18 ejaculates)

The data were analysed and the analysis of variance is given in Table V.

TABLE V

Analysis of Variance

Source	*d. f.*	*M. S.*
Between treatments	3	172341.66†
Between samples	17	51812.66†
Interaction:		
Treatments × samples	51	8731.13
Total	71	—

† Significant at 1% level.

It is apparent that the modifications of the I.V.T. diluent containing 0.9%, or 1.2% glucose were superior to the diluent containing 0.3% or

0.6% glucose. The differences were statistically significant. However, the differences between the diluents containing 0.3% and 0.6% or 0.9% and 1.2% glucose were not significant. The differences between samples were highly significant.

The results of the present investigation revealed that at low dilution (1 : 20), increasing levels of glucose upto 1.2% progressively improved the preservability of bull spermatozoa at room temperatures in the I.V.T. diluent.

Summary

Results of studies with semen from four bulls of *Hariana* breed of cattle, preserved in the I.V.T. diluent for seven days indicate that :

1. In this diluent, *Hariana* bull semen can be preserved over wide range of temperatures from 4-34°C. However, in this range better results are obtained when semen is stored at lower temperatures.

2. In the absence of KCl, the spermatozoa were stored better at a dilution of 1 : 20 compared to a dilution of 1 : 200.

3. At lower dilution level, KCl is not required; but, at higher level of dilution, preservation is improved by adding 0.04% or 0.08% KCl to the diluent.

4. At the dilution level of 1 : 20, a glucose concentration of 0.9% or 1.2% significantly improved the preservability of spermatozoa than a concentration of 0.3% or 0.6%.

Acknowledgement

The authors wish to express their gratitude to Shri P. G. Pande, M.Sc., M.R.C.V.S., Director, Indian Veterinary Research Institute for his interest and encouragement. Sincere thanks are also due to Dr. S. S. Prabhu, Ph.D., D.Sc., Head of Division of Animal Genetics for his advice and for kindly vetting the paper and to Shri R. S. Ganeshan, Assistant Research Officer (Statistics) for advice in statistical treatment of the data.

References

Becze, J. 1950 The storage of bull semen at room temperature. *Animal Breed. Abstr.* **28** : 1927.

Blackshaw, A. W. 1953a The motility of ram and bull spermatozoa in dilute suspension. *J. Gen. Physiol.* **36** : 449-462.

Blackshaw, A. W. 1953b The effect of potassium and calcium salts on the motility of ram, rabbit and bull spermatozoa. *J. Physiol.* **120** : 465-470.

Cragle, R. C. and Salisbury, G. W. 1959 Factors influencing metabolic activity of bull spermatozoa. IV. pH, osmotic pressure and the cations, sodium, potassium and calcium. *J. Dairy Sci.* **42** : 1304-1313.

Jaskowski, L. 1958 Investigations on the preservation of bull semen III. A diluent for the preservation of bull semen at an inconstant temperature. Preliminary data. *Animal Breed. Abstr.* **26** : 1342.

Kampschmidt, R. F., Mayer, D. T , Herman, H. A. and Dickerson, G. E. 1951 Viability of bull spermatozoa as influenced by electrolyte concentration, buffer efficiency and added glucose in the storage media. *J. Dairy Sci.* **34** : 45-51.

Lardy, H. A., and Phillips, P. H. 1943 Effect of *p*H and certain electrolytes on the metabolism of ejaculated spermatozoa. *Am. J. Physiol.* **138** : 741-746

Macleod, J. 1941 Sperm motility and glycolysis. *Endocrinology.* **29** : 583-591.

Mahajan, S. C. and Sharma, U. D. 1961 Comparative efficacy of egg yolk buffer diluents containing carbon dioxide for the preservation of bull semen. J. Anim. Morph. Physiol. **8** : 2, 33-39.

Salisbury, G. W. and VanDemark, N. L. 1945 Stimulation of livability and glycolysis by addition of glucose to egg yolk citrate diluent for ejaculated bovine semen. *Am. J. Phpsiol.* **143** : 692-700.

Scott, J. V. and Hardenbrook, H. J. 1958 Studies on the use of Illini Variable Temperature semen diluent in beef cattle. *J. Amer. Vet. Med. Assoc.* **133** : 432-434.

Senegacnik, J. 1958 A contribution to the study of the effect of CO_2 on the viability of bull spermatozoa. *Animal Breed. Abstr.* **27** : 1357.

Sharma, U. D. 1957 The presevation of bovine semen at room temperature. Ph.D. thesis, Illinois University.

Sharma, U. D. 1960 The preservation of bovine semen at room temperature. *J. Anim. Morph. Physiol.* **7** : 37-54.

Sharma, U. D. and Mahajan, S. C. 1960 Some observations on the preservation of buffalo semen in the Illini Variable Temperature diluent. (Under publication).

Smith, J. T., Mayer, D. T. and Herman, H. A. 1953 Osmotic pressure of extended bovine semen. *Mo. Agr. Expt. Research bull.* No. 538.

Smith, J. T., Mayer, D. T. and Herman, H. A. 1954 A comparison of the ability of certain egg yolk dilutors to maintain optimum osmotic conditions during the storage of bull semen. *J. Dairy Sci.* **37** : 684-690.

VanDemark N. L. and Sharma, U. D. 1957 Preliminary fertility results from the preservation of bovine semen at room temperatures. *J. Dairy Sci.* **40** : 438-439.

White, I. G. 1953 Metabolic studies of washed and diluted ram and bull spermatozoa. *Australian J. Biol. Sci.* **6** : 706-715.

[Received for publication on 24th March, 1961]

SOME OBSERVATIONS ON THE EFFECT OF PARTIAL HEPATECTOMY ON THE COMPOSITION OF BLOOD AND THE REGENERATING LIVER IN LABORATORY RATS

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REMOVAL of the liver substance may be regarded as a "depletion stimulus" to the ensuing proliferative process. This affords an opportunity for a quantitative study of the substances of metabolic significance in the regenerating and the restorative processes, (Drabkin, 1947). The results of a study conducted to find out the chemical composition of blood and liver in partially hepatectomized rats, are presented here.

Materials and Methods

White adult albino rats weighing about 200 gm. were used. Records regarding their preoperative weights, postoperative weights etc. were kept. A twenty four hour fasting period was maintained both before the operation and the sacrifice of the animals, after 8 days of the postoperative period. The median and left lateral lobes were removed in the operation. The portion of the liver tissue thus removed and analysed served as control. In addition to this, a second group of control animals from the same littermates, was kept along with the experimental group. The animals of this control group were given the same diet, which consisted of 18 percent of proteins, as was taken by the animals of the experimental group. The rats belonging to the second control group were sacrificed by decapitation at the same time as those of the experimental group were. Blood samples were collected for analysis.

The water content of the liver was determined. The ratio of wet weight to dry weight was calculated. The total protein content and protein-fractions were determined in the liver tissue extracts and in the plasma samples by the methods previously reported (Pai, 1953). The electrophoretic separation of the plasma proteins was done by paper electrophoresis and their quantitative estimation was done by means of a densitometer, as reported in a previous communication (Pai, 1958), to compare the results of chemical analysis. The total lipid in the liver tissue

[J. Anim Morph. Physiol., Vol. 8, No. 2, pp. 117-125, 1961]

and in plasma were estimated by following the method of Folch (1951). The results of these analysis are shown in the tables and are represented graphically in the chart.

TABLE I

Table showing composition of normal and regenerated liver tissue

Normal

Sr. No.	gm./100 gm. of liver tissue (wet weight)					
	Moist.	Wet : dry	T. P.	Al.	Gl.	T. L.
1	63.00	2.70	10.90	5.00	5.90	6.00
2	63.00	2.70	11.30	5.90	5.40	6.00
3	62.50	2.67	11.20	5.90	5.30	5.20
4	63.00	2.70	10.60	4.90	5.70	4.40
5	65.00	2.86	9.65	5.51	4.14	4.20
6	64.50	2.81	10.60	5.39	5.21	4.00
7	67.00	3.01	9.00	4.70	4.30	3.90
Average	64.00	2.78	10.46	5.33	5.13	4.80
	Regenerated					
1	74.50	3.92	7.63	3.80	3.83	5.00
2	75.00	4.00	8.52	4.40	4.12	4.00
3	70.00	3.33	6.60	2.82	3.78	2.80
4	69.50	3.30	8.65	4.50	4.15	3.90
Average	72.25	3.64	7.84	3.88	3.72	3.90

T. P. = Total proteins Al. = Albumin
Gl. = Globulin T.L. = Total Lipids

TABLE II

Table showing composition of normal and regenerated liver tissue on dry weight basis

Normal

Sr. No.	Moist.	gm./100 gm. of liver tissue (dry weight)				
		Wet. dry	T. P.	Al.	Gl.	T. L.
1	63.00	2.70	29.70	13.50	15.90	16.20
2	63.00	2.70	30.05	15.90	14.60	16.20
3	62.50	2.67	29.90	15.75	14.15	13.90
4	63.00	2.70	31.30	13.25	15.40	11.90
5	65.00	2.86	28.50	15.80	11.80	12.00
6	64.50	2.81	29.80	15.75	14.65	11.25
7	67.00	3.01	27.40	14.15	12.95	11.75
Regenerated						
1	74.50	3.92	29.95	14.90	14.50	19.60
2	75.00	4.00	33.08	17.60	15.40	16.00
3	70.00	3.33	22.00	9.40	12.60	9.35
4	68.50	3.30	28.80	14.85	13.20	12.55

TABLES III—(a) and (b), IV—(a) and (b)

Tables showing the average composition of normal and regenerated liver tissue on wet weight and dry weight basis

***III*—(a) Normal**

	Moist	Wet : dry	gm./100 gm. of liver tissue (wet weight)			
			T. P.	Al.	Gl.	T. L.
Maximum	67.00	3.01	11.30	5.90	5.90	6.00
Minimum	62.50	2.67	9.00	4.70	4.14	3.90
Mean	64.00	2.78	10.46	5.33	5.13	4.80

III—(b) **Regenerated**

Maximum	75.00	4.00	8.65	4.50	4.15	5.00
Minimum	68.50	3.30	6.60	2.82	3.78	2.80
Mean	72.25	3.64	7.84	3.88	3.72	3.90

IV—(a) **Normal**

			gm./100 gm. of liver tissue (dry weight)			
Maximum	67.00	3.01	31.30	15.90	15.90	16.20
Minimum	62.50	2.67	27.40	13.25	11.80	11.25
Mean	64.00	2.78	29.10	14.80	14.20	13.30

IV—(b) **Regenerated**

Maximum	75.00	4.00	34.08	17.60	16.48	19.60
Minimum	68.50	3.30	22.00	9.40	12.60	9.35
Mean	72.25	3.64	28.50	14.10	13.60	14.20

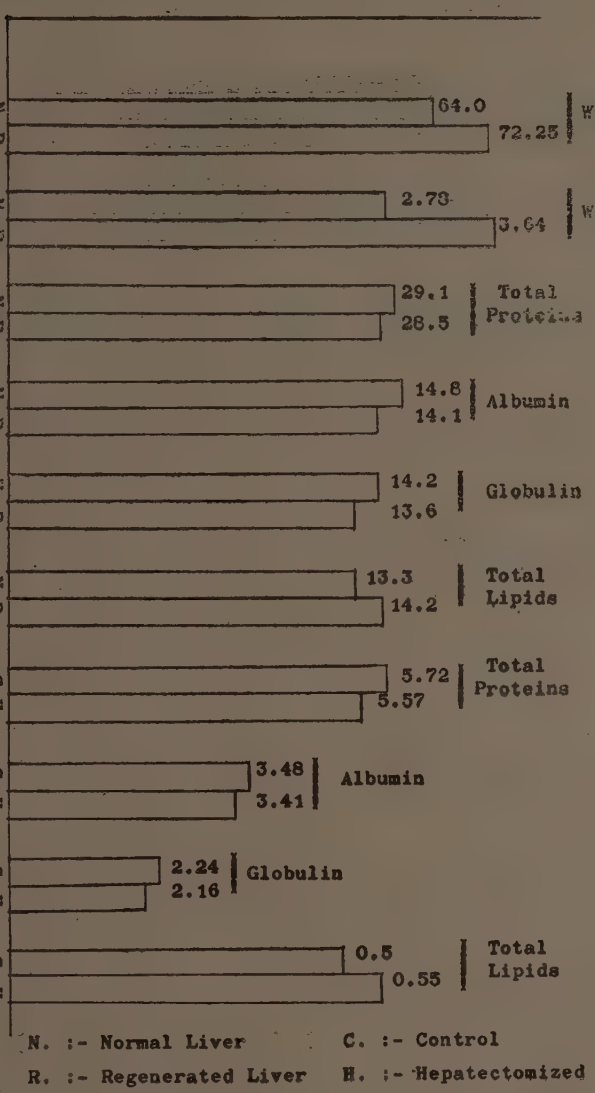
TABLE V

Table showing composition of blood in control and hepatectomized rates

Normal

	gm./100 c. c. of plasma			
	T. P.	Al.	Gl.	T. L.
Average	5.72	3.48	2.24	0.50
Hepatectomized				
Average..	5.57	3.41	2.16	0.55

T. P. = Total proteins Al. = Albumin
Gl. = Globulin T. L. = Total Lipids



Composition of normal and regenerated liver tissue and of blood in control and hepatectomized rats. Figures indicate composition of tissue in gm. % of dry weight and composition of plasma in gm/100 cc.

Results and Discussions

The average food intake of the animals was 10 gm. per day. The average gain in weight of the animals in the control group was 3 gm. per day and that for the animals in the experimental group was 1.5 gm. per day. The average liver weight in terms of percentage of body weight for the animals in the control group was 5.0, the average percentage of liver excised at the time of partial hepatectomy was 60 and the average percentage of liver restored during the period of 8 days after operation, as calculated, was 83. These figures for percentage of restoration of the liver tissue after the partial hepatectomy obtained in this series are comparable to the figures given by other workers (Sutherland, 1956; Mehrotra *et al*, 1956; Mangalik *et al*, 1954 and Newman *et al*, 1951). In the latter studies, some observations on the effect of thyroid gland or the other hormonal effects on the regeneration of liver in rats and such laboratory animals have been reported. Tsubot, Yokoyama, Stowell and Wilson (1954) reported the chemical composition of regenerating mouse liver following partial hepatectomy. The authors remarked that in spite of the existence of an extensive literature dealing with chemical studies on regenerating liver, much of the available information is not readily comparable owing to a lack of uniformity in the experimental conditions, methods etc. employed.

In the present study an attempt has been made to present a correlated chemical picture of regenerative growth as observed in rat liver following partial hepatectomy. In table No. 1, 2, 3 and 4 is shown the composition with respect to total proteins, protein fractions, total lipids etc. of the normal liver tissue from the animals of the control group against those of the regenerated tissue of the animals from the experimental group. In the case of the control group, the water content in the liver tissue varied from 62.50 to 67.00 per cent with an average value of 64.00 per cent. The wet weight to dry weight ratio ranged between 2.67 and 3.01 with a mean value of 2.78. The total proteins ranged from 9.00 to 11.30 gm. per cent on wet weight basis with a mean value of 10.46, whereas on dry weight basis (vide table No. 2) the range was from 27.40 to 31.30 with a mean value of 29.10 gm. per cent. The albumin content on wet weight basis ranged from 4.70 to 5.90 gm. per cent with a mean value of 5.33 gm. per cent, whereas on dry weight basis the range was from 13.25 to 15.90 with a mean value of 14.8 gm. per cent. The values for

globulin varied between 4.14 and 5.90 with a mean value of 5.13 gm. per 100 gm. of wet weight of tissue, whereas the range was from 11.80 to 15.90 with a mean of 14.20 gm. per 100 gm. of dry weight of tissue. The total lipid content on wet weight basis varied from 3.90 to 6.00 with a mean value of 4.80 gm. per cent, whereas the range on dry weight basis was from 11.25 to 16.20 with a mean value of 13.30 gm. per cent.

Similarly the composition of regenerated tissues, which has been studied, (vide tables 1 and 2), is as follows. The water content as well as the wet weight to dry weight ratio are higher than those in the normal tissue. The water content in regenerated tissue varied from 68.50 to 75.00 per cent with a mean value of 72.25 and wet weight to dry weight ratio ranged from 3.30 to 4.00 with an average value of 3.64. The total proteins on wet weight basis ranged from 6.60 to 8.65 gm. per cent with an average of 7.84, whereas on dry weight basis the range was from 22.00 to 33.08, with an average value of 28.5 gm. per cent. The albumin content varied from 2.82 to 4.50 with an average of 3.88 gm. per 100 gm. of wet weight of tissue, whereas it ranged between 9.40 and 17.60 with a mean value of 14.10 gm. per 100 gm. of dry weight of tissue. The globulin varied from 3.78 to 4.15 with a mean of 3.72 gm. per 100 gm. of wet weight of tissue, while it ranged between 12.60 and 15.40 with an average value of 13.60 gm. per 100 gm. of dry weight of tissue. The range for total lipid content on the wet weight basis was from 2.80 to 5.00 with a mean value of 3.90 gm. per cent, while on the dry weight basis the range was from 9.35 to 19.60 with a mean value of 14.2 gm. per cent.

The composition of blood, with respect to total proteins, albumin, globulin and total lipids, in normal and hepatectomized animals has been shown in table No. 5. The total proteins averaged as 5.72 gm. per cent in normal animals while in hepatectomized animals they averaged as 5.57 gm. per cent. The mean values of albumin, globulin and total lipids in normal animals were 3.48, 2.24 and 0.50 gm. per cent respectively, whereas in hepatectomized animals the corresponding values were 3.71, 2.16 and 0.55 gm. per cent. These results have been graphically represented in chart (vide chart No. 1). It will be seen from this chart and the tables, that the regenerated tissues contained a larger amount of water and consequently show the higher wet weight to dry wet ratio than the normal tissues. The composition with respect to tatal proteins, albumin, globulin and total lipid content in normal tissues was higher than in the

regenerated tissue when considered on wet weight basis, which could be explained because of the higher water content in the latter than in the former. The regenerated tissue, however, seems to be almost nearing in its composition to that of the normal tissue at the end of the postoperative period of 8 days after the partial hepatectomy, as can be seen from the chart showing the composition of the tissues on the dry weight basis. In other words the chemical constituents, like total proteins, albumin, globulin and total lipids which have been studied here in the present series, are being restored to normal or nearly normal level during the period of 8 days studied after the operation for partial hepatectomy. The composition of blood in the normal and in hepatectomized animals when compared with the composition of the liver tissue, both normal and regenerated on the dry weight basis, shows that these chemical constituents in the blood of hepatectomized animals are also approaching the normal level, thus resembling the pattern which was seen in the case of the composition of the regenerated tissue as compared to that of normal liver tissue, on dry weight basis.

Summary and Conclusions

1. Some of the changes in the chemical composition of the liver during regeneration in laboratory rats after partial hepatectomy have been studied.

2. Changes in the chemical composition of the blood were also observed and compared with those in the liver tissue during regeneration.

3. It has been observed after comparing the composition of regenerated tissue with that of the normal liver tissue on dry weight basis that the chemical constituents *e. g.* total proteins, albumin, globulin and total lipids were restored to normal or nearly normal during the period of 8 days after the operation for partial hepatectomy.

4. On comparing the composition of blood of normal and of hepatectomized animals, with the composition of liver tissue, normal and regenerated, it was found that the blood constituents studied were coming to normalcy, as was seen in case of the regenerated liver tissue.

Acknowledgement

Thanks are due to the Dean, Medical College, Baroda, for permission granted, to the Professor of Physiology, for the facilities given, to the M. S. University of Baroda, for financial aid and to the members of staff in the laboratory for their co-operation.

References

Drabkin, D. L. 1947. Liver regeneration and cytochrome C metabolism. Influence of amount of tissue excised and of diet, with a note on accompanying changes in liver nucleic acids. *J. Biol. Chem.* **171** : 395.

Folch, J. ; Arsove, S. and Meath, J. A. 1951. Preparation of lipid extract from brain tissue. *J. Biol. Chem.* **191** : 833.

Mangalik, V. S. ; Mehrotra, R.M.L. and Agarwal, K. C. 1954. Role of thyroid gland in liver regeneration. *Ind. Jour. Med. Sci*, **8** : 477.

Mehrotra, R.M.L. and Mangalik, V. S. 1956. An experimental study of the effect of cortisone, DOCA and adrenalectomy on regeneration of liver after partial hepatectomy in albino rats. *Ind. Jour. Med. Res.* **44** : 325.

Newman, E. A. and Grossman M. I. 1951 Effect of Nucleic acid supplements in the diet on the rate of regeneration of liver in rats. *Am. J. Physiol.* **164** : 251.

Pai, M. L. 1953. Plasma protein fractions in oedema and ascites. *Jour. M. S. Univ. Baroda*, **2** : 29.

Pai, M. L. 1958. Study of Plasma proteins by electrophoresis in diseases. *J Anim. Morph. Physiol.* **5** : 90.

Sutherland, A. M. 1956 Regeneration in the fatty liver of the rat after partial hepatectomy. *J. Path. Bact.* **71** : 403.

Tsubot, K. K. ; Yokoyama, H. O. ; Stowell R E. and Wilson, M. E. 1954. The chemical composition of regenerating mouse liver. *Arch. Biochem.* **48** : 275.

[Received for publication on 2nd, January, 1961]

THE RELATIVE CONTENT OF IMINO ACIDS IN THE SKIN AND SCALES OF SOME TELEOSTS

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AN interesting aspect of the organic composition of the skin and scales of fish relates to imino acids. In recent years the investigation of the hydroxyproline content of the fish skin has assumed significance in relation to the thermal stability of its collagen. Gustavson (1956) and Takhashi have shown that the shrinkage temperatures of skin collagen of 'cold water' fishes are lower than those of 'warm water' fishes. As Anfinsen (1960) stated, nature seems to have devised collagen molecules suited to cold or warm habits through the introduction or deletion of hydroxyproline residues. Very recently Piez (1960) and Piez et al. (1960) have presented evidence to indicate that the varying stabilities exhibited by collagens are related to the pyrrolidine ring content rather than the hydroxy group of hydroxyproline and *i. e.* the total hydroxyproline and proline content that is important in shrinkage.

Almost all the studies made so far on hydroxyproline in fishes relate to either the skin collagen or scale gelatin only. A few investigations (Solomons. 1955; Burley and Solomons 1957) give the imino acid values for ichthylepidin of the scales.

Since scales develop from the skin, it would be interesting to know the relative imino acid content of the scales and skin. The present authors have been studying the composition and regeneration of fish skin and scales. In this context, the relative content of imino acids of the skin and scales has been determined in a few fishes. The following account gives the values of the imino acids for nine species of teleosts.

Material

The hydroxyproline and proline content of the skin and scales of the following fish was determined.

[J. Anim. Morph. Physiol., Vol. 8, No. 2, pp. 126-129]

1. *Hilsa toli* (C. V), 2. *Opisthopterus tardoore* (Cuv), 3. *Sardinella albella* (Val), 4. *Mugil cephalus* Linn, 5. *Polynemus tetradactylus* (Shaw), 6. *Ophiocephalus striatus* Bloch, 7. *Boleophthalmus boddaertii* Pall, 8. *Glossogobius giurius* (Ham-Buch), 9. *Acentrogobius viridipunctatus* (Val).

Analytical Procedure

Specimens of the fish were obtained fresh and immediately prepared for analysis.

Hydrolysis :—0.05 gm. of tissue dried to constant weight was hydrolysed with 10 ml. of 6N HCl for 18 hours under reflux in a water bath.

Imino Acid analysis : The method of Neuman and Logan modified by Leach (1960) was adopted for hydroxyproline determinations. This method gives a greater reproducibility amongst replicates and improves colour yields reproducible over prolonged periods.

For the determination of proline, the colorimetric method described by Troll and Lindsley (1955) was adopted. In this procedure the interfering basic amino acids, lysine and hydroxylysine, are removed by shaking the solutions with permutit. The procedure is entirely specific for proline.

The colorimetric estimations were carried out using a Klett-Summerson photoelectric colorimeter with filter No. 54.

For the determination of nitrogen in the skin and scales, the microkjeldahl (Hawk et al. 1954) method was adopted. The protein in skin and scales was calculated by multiplying the nitrogen value with 6.025 as in an earlier investigation by the authors (1960) and as discussed by Love (1957).

Results

As the aim of the present study is to know the content of the imino acids in the skin and scales, and not of collagen and ichthylepidin separately, the values are expressed as percentage of protein in the skin and scales. The results obtained are presented in Table 1.

TABLE I

	Hydroxy-Proline gm. imino acid/100 gm. Protein		Proline gm. imino acid/100 gm. Protein		Hy. Proline + Proline	
	Skin	Scales	Skin	Scales	Skin	Scales
Hilsa toli (C. V.)	6.04	8.97	10.9	12.2	16.94	21.17
Opisthopterus tardoores (Cuv.) ..	5.11	8.78	8.96	12.65	14.07	21.43
Sardinella albella Val	5.66	7.15	8.92	11.50	14.58	18.65
Mugil cephalus Linn	5.13	7.38	10.10	10.70	15.23	18.08
Polynemus tetradactylus (Shaw) ..	6.50	6.61	12.10	13.30	18.60	19.91
Ophiocephalus striatus Bloch ..	6.04	7.35	9.48	12.93	15.52	20.28
Boleophthalmus boddaerti Pall ..	5.81	6.82	8.10	9.63	13.91	16.45
Glossogobius giuris (Ham-Buch)..	6.02	8.37	10.20	12.15	16.22	20.52
Acentrogobius viridipunctatus (Val)	6.26	9.20	9.68	14.10	15.94	23.30

Discussion

As mentioned already, the relative content of imino acids in the skin and scales has not been studied by previous investigators. From the present study it is seen that the proline and hydroxyproline values are higher for the scales than for the skin in all the species studied.

With the limited number of determinations made in the present study it is not possible to establish a functional relationship between the imino acids content of the scales and skin. However, there is some indication of a *statistical relationship* between the scales and skin in respect of the total of hydroxyproline + proline values, when plotted graphically. The separate values do not indicate this trend.

According to Piez *et al.* (1960) the number of hydroxyproline residues in the carp gelatin is slightly less in the scales than in the skin, but the proline residues are slightly higher in the skin, and the total hydroxyproline + proline residues in the scale gelatin and skin collagen of the carp is almost the same.

The scales of most teleosts contain two proteins (1) collagen which is convertible to gelatin, and (2) ichthylepidin which is insoluble in boiling water, cold dilute acids and alkalis. In the present study the imino acid content of the scale proteins as a whole has been determined besides the skin imino acids. The study of hydroxyproline and proline residues

separately for ichthylepidin, gelatin of the scale and collagen of skin in different teleosts would be of much interest and is now engaging the attention of the authors.

Summary

The relative proline and hodroxyproline content in the scales and skin of nine species of teleosts has been determined. The values of these imino acids are higher in the scales than in the skin.

Acknowledgment

Our thanks are due to Prof. R. V. Seshaiya, Director, Marine Biological Station, Porto Novo for instruction and guidance and to the University Grants Commission for the award of Research Scholarships to both of us.

References

Ambuja bai, P. & Kalyani, M 1960 On the composition of the scales and skin of *Boleophthalmus boddaertii*Pall and *Mugil dussumieri* (Val). *J. Anim. Morph. Physiol.* **7**: 71.

Anfinsen, C. B. 1960 The Molecular Basis of evolution p. 215. John Wiley and Sons. Inc. Publishers.

Burley, R. W. & Solomons, C. C. 1957 The amino acid composition of fish scale proteins. *S. Afr. industr. Chem.* 154.

Eastoe, J. E. 1957 The amino acid composition of fish collagen and gelatin. *Biochem. J.* **65**: 363.

Gustavson, K. H. 1956 The chemisiry and reactivity of collagen. p. 78. Academic Press Inc. New York. The analytical studies of Takahshi discussed on pp. 224-227.

Hawk, P. B., Oser, B. L. and Summerson, W. H. 1954 Practical physiological chemistry (13th edition) p. 644 Mc Graw Hill Book Co. Inc., New York.

Leach, A. A. 1960 Notes on a modification of the Neuman and Logan method for the determination of hydroxyproline. *Biochem. J.* **74**: 70.

Love, R. M. 1957 The physiology of fishes. Ed. Brown. M.B., **1**: 403. Academic Press Inc. New York.

Neuman, R E. 1949 The amino acid composition of gelatins, collagens and elastins from different sources. *Arch. Biochem.* XXIII—XXIV, 289.

Piez, A. K. 1960 The relation between Amino acid composition and denaturation of vertebrate collagens. *J. Amer. chem. Soc.* **82**: 247.

Piez, A. K. & Groos, J. 1960 The amino acid composition of some fish collagens. Relation between structure and composition. *J. biol.* chem. **235**: 995.

Solomons, C. 1955 Protein in Fish Scale *S. Afr. J. Med. Sci.* **20**: 27.

Troll, W. & Lindsley, J. 1955 A photometric method for the determination of proline. *J. biol. Chem.* **215**: 655.

Winter, H. 1954 Zur Kenntnis des Ichthylepidins, der Gerüstubstanz der fischschuppen. *Archiv für Fischereiwissenschaft.* 170.

[Received for publication on 7th April, 1961]

NEUROSECRETORY CELLS IN THE BEETLE, HELIOCOPRIS BUCEPHALUS F.

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Kopec (1917, 22) showed that the pupation in the full grown caterpillar is induced by a hormone from the brain. This fundamental discovery gave an incentive for further work on neurosecretion in insects. The function of hormone secretion in insects was later ascribed to certain cells in the central nervous system. These cells on account of their glandular activities are now known as neurosecretory cells.

The structural peculiarities and the functional significance of neurosecretory cells in insects have been discussed by Wigglesworth (1934, 36, 52); Weyer (1935); Fraenkel (1935); Hanstrom (1938, 53); Scharrer (1941, 52b, 53, 54); Williams (1952); Thomsen, E. (1952); Arvy and Gabe (1953); Nayar (1953, 54, 55, 56, 58); Karlson (1957); and Highnam (1958).

The present account on the structure of neurosecretory cells in the beetle *Heliocopris bucephalus F.* is given as they show certain interesting variations in the brain, thoracic and abdominal ganglia.

Materials and Methods

Beetles were collected at night under the street lights and they were then dissected in Ringer's solution. Brain, nerve cord, thoracic and abdominal ganglia were removed and fixed in Bouin's fluid, 2% formalin and Helly's fixative. Of these alcoholic Bouin's gave good results.

Material thus obtained was embedded in paraffin and serial sections were cut at 4μ. Gomori's chrome-alum-haematoxylin-phloxin, Heidenhain's Azan and Haematoxylin-eosin stains were used.

Of these, Gomori's chrome-alum-haematoxylin-phloxin and Heidenhain's Azan gave satisfactory results. Serial sections were then examined under microscope and photomicrographs were taken.

[J. Anim. Morph. Physiol., Vol. 8, No. 2, pp. 130-136, 1961]

Observations

Neurosecretory cells are present in the brain, thoracic and abdominal ganglia but they are absent in the nerve cord. They can easily be distinguished from the nerve cells by their characteristic large sizes, their nuclei, vacuoles and staining properties.

Cytoplasm of the neurosecretory cells appears brilliant blue when stained with chrome-alum-haematoxylin-phloxin and shows a reddish tinge in Azan.

Under the microscope three types of neurosecretory cells have been identified on the basis of their structural peculiarities and staining properties. They have been designated as A, B and C types.

A—Cells (Pl. 1, Figs. D and E)

A—cells are present either singly or in groups in the brain, thoracic and abdominal ganglia and are the largest of the three types measuring between 32μ and 100μ in diameter. The cells in the thoracic ganglion are relatively more prominent as compared with those of brain and abdominal ganglia on account of their large sizes.

A—cells show considerable variation in shape but they can be easily identified on the basis of their deeply stained granular and vacuolated cytoplasm. The vacuoles are generally observed at the periphery of the cell, though at times they may be observed near the centrally situated nucleus.

The nucleus is spherical in shape with a sharply defined nuclear membrane. Inside the nucleus the chromonemata are loosely dispersed and can be recognised by their deep red colour in both Gomori's and Azan stains.

B—Cells (Pl. 1, Fig. F.)

The B—cells are oval in shape and relatively smaller than the A cells, with diameters ranging between 16μ and 40μ. Each cell shows characteristic dense granular cytoplasm concentrated around the nucleus and a conspicuous vacuole. The vacuole is situated just inside the cell boundary and almost surrounds the cytoplasm and nucleus. The chromatin material of the nucleus is in the form of deeply stained small bodies, which are disposed along the inner face of the nuclear membrane. The direction of flow of secretory material formed inside the cell is towards the point where the axon originates. The secretory material has also

been traced into the axon. This type of cell is present only in the thoracic and abdominal ganglia. It is absent in the brain.

C—Cells (Pl. 1, Fig. G.)

C—cells are oval in shape with distinct cell outlines and their diameters range between 16μ and 36μ. They are characterised by the complete absence of vacuoles. The cytoplasm of these cells is usually coarse, but in some cells it shows a homogeneous appearance. The centrally placed nucleus is large in proportion to the size of the cell, with large deeply staining chromatin blocks. This type is represented in brain, thoracic and abdominal ganglia.

The distribution and arrangement of the neurosecretory cells in the brain, thoracic and abdominal ganglia is very characteristic. The brain shows only the A and C types of neurosecretory cells, the latter being more numerous. They are more or less distributed on the periphery of brain and show slight variations in size, number and arrangement in its different regions. In the anterior region of the brain they are few in number and small in size and occur medially; while in the central and posterior regions they are comparatively larger and take up medial as well as lateral positions (Pl. 1, Fig. A). In all these three regions the concentration of the cells is more on the ventral as compared to the dorsal region of the brain. It may also be mentioned that the total number of cells is greater in the central and the posterior regions than in the anterior region of the brain. Moreover, they are much smaller than those of the thoracic and abdominal ganglia.

In the thoracic ganglion all the three types of neurosecretory cells are represented. They are comparatively larger in size and also greater in number than those in the brain. The distribution and arrangement of

Plate 1 :—Photomicrographs of prepared sections of brain, thoracic and abdominal ganglia of *Heliocopris bucephalus F.*

Figures :—A Transverse section of the brain showing the median and lateral neurosecretory cells.

B Transverse section of the thoracic ganglion showing the distribution of neurosecretory cells.

C Transverse section of the abdominal ganglion showing the distribution of neurosecretory cells at the periphery.

D A—Cell from the thoracic ganglion showing the vacuoles at the periphery.

E A—Cells in a group.

F B—Cells in a group showing large vacuoles.

G C—Cells showing distinct cell outlines, and large nuclei.

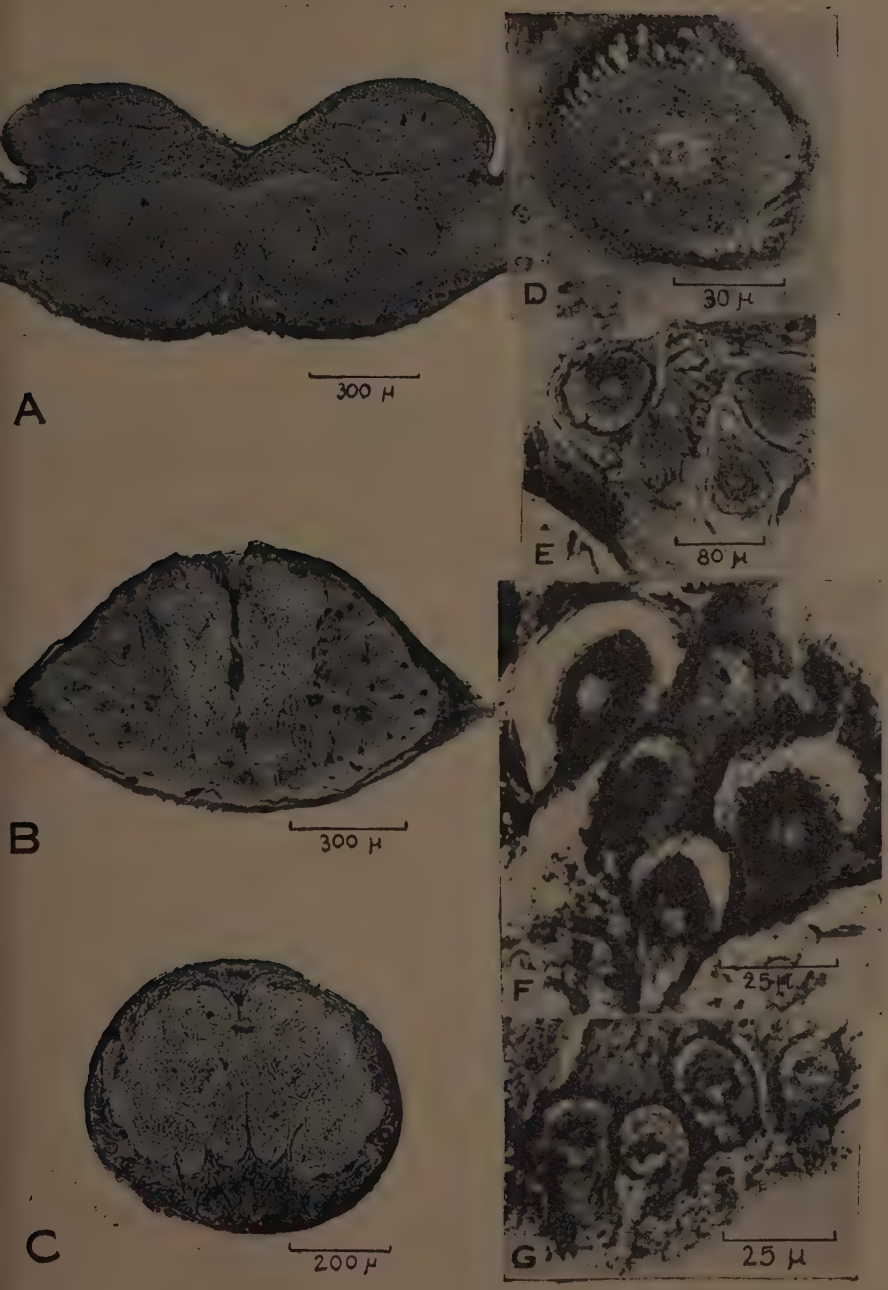

PLATE 1

these cells is similar to those of the brain (Pl. 1, Fig. B). The A cells are found either singly or in groups but the B and C cells are as a rule found in groups in the thoracic and also the abdominal ganglia.

The abdominal ganglion shows all the three types of cells which are located in its peripheral region (Pl. 1, Fig. C). The B and C cells show a regular distribution in the anterior, central and posterior regions of the ganglion. The A-type, however, is common in the central and posterior regions but is quite rare in the anterior region. Their arrangement as seen in the section (Pl. 1, Fig. C) is similar to that of the brain and the thoracic ganglion.

Discussion

The distribution of neurosecretory cells in the central nervous system of *Heliocopris bucephalus F.* resembles on the whole that of the fruit fly *Chaetodacus cucurbitae-Cog.* (Nayar, 1954). But in *Heliocopris bucephalus* it has been possible to identify three distinct types of neurosecretory cells namely A, B and C. All the three types of cells are represented in the thoracic and abdominal ganglia. However, in the brain, B-type is absent while the other two types are present.

The A-type of cell shows numerous small vacuoles and their number on the average is twenty in each cell. The B-type of cell possesses a single large vacuole extending over a major area of the cell. Presence of vacuoles indicates a high degree of activity on the part of neurosecretory cells; wherein there is a constant elaboration followed by quick withdrawal of neurosecretory material. In the A-type of cell the numerous small vacuoles appear to converge on the origin of axon ; while in the B-type the single large vacuole extends into the axon. These observations suggest that the neurosecretory colloids formed inside the cell are transported through the axon. These observations on the axonic transport of the secretory material, support the earlier findings by Scharrer (1952b) in *Leucophaea ;* Arvy, Bounhiol and Gabe (1953) in *Bombyx ;* Arvy and Gabe (1954) in *Plecoptera ;* E. Thomsen (1954) in *Calliphora* and M. Thomsen (1954) in *Hymenoptera.* The C-type of cell shows complete absence of vacuoles but at the same time neurosecretory material is observed flowing into the axon of this cell. On the basis of the number, size and disposition of vacuoles present in the cells it can be surmised that the degree of activity in the B cells is higher than that of the A-cells. Since the C-type of cells do not show any vacuoles it is suggested that its degree of activity is the lowest.

Summary

Distribution of neurosecretory cells in the brain, thoracic and abdominal ganglia of *Heliocopris bucephalus F.* is described. On the basis of the structure and staining properties, it has been possible to identify three types of neurosecretory cells; and they have been designated as A, B and C types.

All the three types of neurosecretory cells are observed in the thoracic and abdominal ganglia, the brain, however, shows only A and C types of cells.

On the basis of the number, size and disposition of vacuoles present in the neurosecretory cells it is suggested that the B-type of cell is more active than the A-type. In view of the absence of vacuole in the C-type of neurosecretory cell it is surmised that its degree of activity is the lowest.

Acknowledgements

Grateful acknowledgement is made to the Government of Maharashtra for making available facilities for these investigations carried out at Vidarbha Mahavidyalaya, Amravati. One of the authors Mr. H. B. Nirmal expresses his gratitude to the Deputy Director of Education Vidarbha, Nagpur for the award of a Research Fellowship. Thanks are also due to Mr. P. B. Sabnis, M.Sc. for his valuable help in photomicrography.

References

Arvy, L., Bounhiol, J. J. & Gabe, M., 1953. *C. R. Acad. Sci.* Paris, **236**, 627.
Arvy, L. & Gabe, M., 1953. *C. R. Acad. Sci.* Paris, **237**, 844.
————————, 1954. *Biol. Bull.*, **106**, 1.
Fraenkel, G., 1935. *Proc. Roy. Soc.* B, **118**, 1.
Haustrom, B., 1938. *Acta. Univ. Lund.*, N. F., Avd. 2, **39**, 1.
————————, 1953. *Nature* **171**, 72.
Highnam, K. C., 1958. *Quart. J. Micr. Sci.*, **99**, 171.
Karlson, P., 1957. *Boll. Zool. Agraria e Bachicoltura*, **22**, 65.
Kopec, S., 1917. As cited by Wigglesworth, V. B., 1957, in "Sympo ia of the society for experimental biology.," number XI, 204., The syndics of the Cambridge University Press, London, N.W. 1.
————————, 1922. *Biol. Bull.*, Woods Hole, **42**, 322.
Nayar, K. K., 1953. *Curr. Sci.*, **22**, 149.
————————, 1953. *Curr. Sci.*, **22**, 241.
————————, 1954. *Proc. Indian Acad. Sci.*, (B), **40**, 138.
————————, 1954. *Quart. J. Micr. Sci.*, **95**, 245.
————————, 1955. *Agra Univ. J. Res. Sci.*, **4**, 419.
————————, 1956. *Quart. J. Micr. Sci.*, **97**, 83.
————————, 1958. *J. Biol. Sci.*, **1**, 26.

Scharrer, B., 1941. *J. Comp. Neurol.*, **74**, 93.
————————, 1952b. *Biol. Bull.*, **102**, 261.
————————, 1953. *Ann. Rev. Phys.*, **15**, 457.
————————, 1954. *Publ. Staz. Zool. Napoli.*, Suppl., **24**, 38.
Thomsen, E., 1952. *J. Exptl. Biol.*, **29**, 137.
————————, 1954. *J. Exptl. Biol.*, **31**, 322.
————————, 1954. *Experientia*, **10**, 206.
Thomsen, M., 1954. *Dan. Biol. Skr.*, **7**, 1.
Weyer, F., 1935. *Zool. Anz.*, **112**, 137.
Wigglesworth, V. B., 1934. *Quart. J. Micr. Sci.*, **77**, 191.
————————, 1936. *Quart. J. Micr. Sci.*, **79**, 91.
————————, 1952. *J. Exp. Biol.*, **29**, 620.
Williams, C. M., 1952. Harvey Lectures, **47**, 126.

[Received for publication on 30th April 1961]

THE OCCURENCE OF A HYPOGLYCEMIC FACTOR IN THE EYESTALKS OF FRESHWATER CRAB, *PARATELPHUSA JACQUEMONTII* (RATHBUN)

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THE role of the endocrine complex in crusta in metabolism has received considerable attention and several reviews on the subject have been published, (Drach, 1939 ; Renaud, 1949 ; Panouse, 1946 ; Kleinholz, 1942 ; Brown, 1944, 1948b and Knowles and Carlisle, 1956). The relation of eyestalk principle and carbohydrate metabolism in crustaceans has been studied earlier and among other papers published on this subject, that of Abramowitz, Hisaw and Papandrea (1944) need special mention since these authors identified a definite structure in the eyestalks of the blue crab, *Callinectes sapidus* which is responsible for the elaboration of a diabetogenic factor. Further evidence in support of the hyperglycemic control mechanism in crustaceans was given by the work of Kleinholz (1948), Kleinholz and Little (1948, 1949), Scheer and Scheer (1951).

Hemmingsen (1924b) and Lindblad (1931) on the other hand suggested that in *Astacus* there is a hypoglycemic regulatory mechanism. The evidence put forward by these authors was, however, indirect and no attempt was made to localize this mechanism.

The present report deals with experiments which clearly point towards the presence of hypoglycemic factor in the eyestalks of the freshwater crab, *Paratelphusa jacquemontii*.

Material and Methods

Crabs were collected from a local river and kept in a container for 24 hours. No feeding was done during this period.

In all 35 animals were used and they were divided into groups as shown in Table 1. A fairly equal distribution of male and female specimens was made in the different groups.

Blood was withdrawn by means of a 1 ml. tuberculin syringe and a hypodermic needle through a small pinhole made in the carapace just

[J. Anim. Morph. Physiol., Vol. 8, No. 2, pp. 137-144 , 1961]

above the pericardial cavity. The carapace was punctured with the help of a dissecting needle. The blood was transferred to a tube and 0.1 ml. was used for the determination of sugar in blood.

The eyestalks from 5 crabs were extirpated and their extract was made in a known quantity of 0.6% saline solution in a mortar. Final volume of the extract was 3 ml. This extract was then subjected to centrifugation and the clear supernatant fluid was used for injection. Sham operation was performed by carefully removing the chitinous plate around the eyestalk. Blood sugar was estimated by the method of Mendel, B., Kemp, A., and Myers, D. K. (1954). Percentage of glycogen and free glucose in hepatopancreas were determined by the method of Kemp, A., Adrienne, J. M., and Kits Van Heijeningen (1954).

Observations

In normal untreated crabs (Table I), average value of blood sugar was 78 mg.% The basal range of the concentration of sugar in this species is (70-85 mg.%) which is higher than that recorded in *Paratelphusa guerinii* (Rangneker, 1955). Average value of free glucose in hepatopancreas was 0.586 mg.% and that of glycogen is 0.556 mg.%.

Extirpation of eyestalks resulted in a marked rise in blood sugar level. The average value was 120 mg.%, and range of fluctuation was from 88-150 mg.%. There was also a decrease in the percentage of free glucose in the hepatopancreas; the average value being 0.355 mg. %. In the control experiment, sham operated animals did not show any appreciable changes in the concentrations of sugar in blood and also those of glycogen and free glucose in the hepatopancreas. The average blood sugar value was 81 mg.% and the average values of glucose and glycogen in the hepatopancreas were 0.578 mg.% and 0.540% mg.% respectively. This experiment was performed to eliminate the possible effect of injury on blood sugar and glycogen levels.

Removal of eyestalks followed by an injection of 0.3 ml. eyestalk extract in saline produced hypoglycemia; the average value of blood sugar being 58 mg.%, and its range 50-70 mg.%. No marked changes were observed in glycogen and free glucose concentrations in the hepatopancreas. In the control experiment ablation of eyestalks followed by an injection of equivalent quantity of saline showed a rise in blood sugar level with a corresponding fall in the glucose level of the hepatopancreas.

TABLE I

Expt. No.	Treatment	No. of Animals	Glycogen in hepato-pancreas in mg.%		Glucose in hepato-pancreas in mg.%		Blood glucose in mg.%	
			Average	Range	Average	Range	Average	Range
1	Normal, Untreated	5	0.556	0.445-0.620	0.586	0.417-0.845	78	70-85
2	(a) Eyes removed: Estimation done after 20 minutes	5	0.666	0.555-0.727	0.355	0.273-0.398	120	88-150
	(b) Sham operation: Chitinous plate around the eyes removed. Estimate done after 20 minutes	5	0.540	0.482-0.596	0.578	0.443-0.738	81	72-84
3	(a) Eyes removed: Injected after 20 minutes with 0.3 ml. extract of eyestalks in 0.6% saline. Estimations done after 20 minutes	5	0.540	0.509-0.632	0.558	0.410-0.673	58	50-70
	(b) Eyes removed: Injected after 20 minutes with 0.3 ml. of 0 6% saline Estimations done after 20 minutes	5	0.563	0.463-0.652	0.302	0.220-0.490	121	85-148

TABLE I(Contd.)

Expt. No.	Treatment	No. of Animals	Glycogen in hepato-pancreas in mg.%		Glucose in hepato-pancreas in mg.%		Blood glucose in mg.%	
			Average	Range	Average	Range	Average	Range
4	(a) Eyes intact: Injected with 0.3 ml. extract of eye-stalks in 0.6% saline. Estimations done after 20 minutes	5	0.783	0.649-0.960	0.577	0.519-0.644	36	32-40
	(b) Eyes intact: Injected with 0.3 ml. of 0.6% saline. Estimations done after 20 minutes	5	0.510	0.493-0.611	0.563	0.486-0.670	75	71-80

The average value of blood sugar concentration was 121 mg.%, while that of glucose in hepatopancreas was 0.302 mg.%. There was no effect on the concentration of glycogen in hepatopancreas. It is evident, therefore, that the fall recorded in blood sugar level with eyestalks removed followed by an injection of eyestalk extract was the effect of hypoglycemic factor present in the extract.

In crabs with intact eyes, injection of eyestalk extract induced a more striking decrease in blood sugar level, the average value being 36 mg.%. In the control experiment, however, injection of an equivalent quantity of saline had no effect on blood sugar concentration, the average value being 75 mg.%. In both these experiments glycogen and glucose concentrations in hepatopancreas did not show any marked changes.

Discussion

Abramowitz *et al.* (1944) demonstrated that injection of eyestalk extracts into crabs of the genus *Callinectes* led to a hyperglycemic condition. They further carried out experiments to see whether removal of eyestalks resulted in hypoglycemia. But their results were paradoxical, as the operated animals showed gradual hyperglycemia, which was attributed to operative injury. Kleinholz and Little (1949) observed in the crab *Libinia emarginata* that eyestalk extract injection resulted in hyperglycemia but removal of eyestalks in that species had no significant effect on blood sugar level. Scheer and Scheer (1951) on the other hand while working on the spiny lobster *Panulirus* demonstrated that eyestalk removal resulted in a fall in blood sugar level, and suggested the possibility that brachyurans differed from the macrurans in this respect.

These earlier findings point towards the presence of a diabetogenic factor in the eyestalks of the decapod crustaceans examined. Our observations are, however, different. In our experiments on the fresh water crab *Paratelphusa*, it was observed that extirpation of eyestalks resulted in a rise in blood sugar level. In control experiments on the other hand the sugar level in sham operated crabs was within a normal range. In a complementary experiment injection of eyestalk extract in eyestalkless crabs resulted in a fall in blood sugar level below normal, while in control experiment after injection of equivalent quantity of saline, there was a rise in blood sugar level as expected. Further more, injection of eyestalk extract in crabs with intact eyes produced a distinct hypoglycemia while in the control experiment with equivalent quantity of 0.6% saline, the

blood sugar remained within a normal range. From these observations it can be concluded ihat in *Paratelphusa*, a hypoglycemic factor is present in the eyestalks and it is concerned with maintaining a constant level of blood sugar. Its action is analogous to that of insulin on blood sugar concentration in higher vertebrates.

It is interesting to note that Hemmingsen (1924) and Lindblad (1931) injected glucose in the crayfish *Astacus* which disappeared from the blood stream too rapidly to have been oxidised to carbondioxide during experimental period. They also observed that no detectable amounts of glucose were excreted in the surrounding water. Scheer and Scheer (1951) confirmed these results in *Panulirus*. These findings suggest that the disappearance of glucose from the blood in the experimental animals is due to the intervention of some hypoglycemic control mechanism (Knowles and Carlisle, 1956) which is yet to be defined. Our experiments on *Paratelphusa* lead to a more specific localization of a hypoglycemic factor in the eyestalks.

In the hepatopancreas of *Paratelphusa* no significant changes in the glycogen concentration were observed following ablation of eyestalks and also injection of eyestalk extract. This may be due to the relatively short experimental period. Neiland and Scheer (1953) have also made similar observations on *Hemigrapsus nudus* following the removal of the sinus gland. Further studies on the effects of the eyestalk principle on glycogen level of the hepatopancreas over an extended experimental period should be worth while.

The amounts of free glucose in hepatopancreas, however, showed some interesting changes under different conditions. Thus, after removal of eyestalks, the glucose concentration in the hepatopancreas was lowered. It remained, however, almost at a normal level after the administration of the eyestalk extract in eyestalkless animals as well as in those with eyestalks. This suggests that there is a mobilization of free glucose in the hepatopancreas following ablation of eyestalks.

Summary

1. Extirpation of eyestalks in *Paratelphusa jacquemontii* resulted in a rise in the sugar concentration of blood, when compared with sham operated controls. Free glucose concentration in hepatopancreas was lowered due to the ablation of eyes.

2. Injection of 0.3 ml. eyestalk extract in eyestalkless animals lowered blood sugar level considerably. This fall in the concentration in the blood sugar represented a hypoglycemic condition. In the control experiment, injection of equivalent quantity of saline in eyestalkless crabs showed a rise in blood sugar concentration as expected, with a corresponding fall in the free glucose level of hepatopancreas.

3. Injection of eyestalk extract in intact animals resulted in a remarkable fall in blood sugar level and the effect was all the more striking than that observed in eyestalkless animals. In the control experiment injection of equivalent quantity of saline had no significant effect on blood sugar level.

4. Eyestalk principle had no significant effect on glycogen level of hepatopancreas.

5. The results obtained suggest that a hypoglycemic factor is present in the eyestalks of *Paratelphusa jacquemontii*.

Acknowledgements

Grateful acknowledgement is made to the Government of Maharashtra for making available research facilities for these investigations carried out at Vidarbha Mahavidyalaya, Amravati. One of the authors P. B. Sabnis, expresses his gratitude to the Deputy Director of Education Vidarbha, Nagpur, for the award of a research fellowship.

References

Abramowitz, A. A., Hisaw, F. L. and Papandrea, D. N.: 1944 The occurrence of a diabetogenic factor in the eyestalks of crustaceans. *Biol. Bull.*, Woods Hole, **86**, 1.

Brown, F. A.: 1944 Hormones in the crustacea. Their sources and activities. *Quart. Rev. Biol.* **19**, 118.

Brown, F. A.: 1948 Hormones in crustaceans. In the hormones, pp. 159-200 (G. Pincus and K. Thimann, ed). New York: Academic Press.

Drach, P.: 1939 Mue et cycle d'intermue chez les Crustaces décapodés. *Ann. Inst. Oceanogr. Monaco*, **19**, 103.

Hemmingsen, A. M.: 1924 Blood Sugar regulation in the crayfise. *Skand. arch. Physiol.*, **46**: 51-55.

Kemp, A., Adrienne, J. M., and Kits Van Heijeningen.: 1954 A Colorimetric Micromethod for the determination of glycogen in tissues. *Biochem. J.* **56**, 646.

Kleinholz, L. H.: 1942 Hormones in Crustacea. *Biol. Rev.* **17**, 91.

Kleinholz, L. H., 1948 Experimental hyperglycemia in the marine crustacean, *Libinia emerginata Anat. Rec.* **101**, 734.

Kleinholz, L. H. and Little, B. C.: 1948 Bleod-sugar values in the marine crustacean, *Libinia emarginata Anat. Rec.* **101**, 734.

Kleinholz, L. H. and Little, B. C.: 1949 Studies in the regulation of blood-sugar concentration in crustaceans. I. Normal values and experimental hyperglycemia in *Libinia emarginata*. *Biol. Bull.*, Woods Hole, **96**, 218.

Knowles, F. G. W., Carlisle, D. B. and Dupont-Raabe, M.: 1956 Endocrine control in the Crustacea. *Biol. Rev.* **31**, 396.

Lindblad, R. G. 1931 Studien über den Blutzucker bei *Astacus fluviatilis*. Kristianstad.

Mendel, B., Kemp, A., and Myers, D. K. 1954 A colorimetric micro-method for the determination of glucose. *Biochem. J.* **56**, 639.

Neilaud, K. A. and Scheer, B. T. 1953 The hormonal regulation of metabolism in crustaceans. V. The influence of fasting and of sinus gland removal on body composition of *Hemigrapsus nudus*. *Phyziol. comp.* **4**, 321.

Panouse, J. B. 1946 Recherches sur les phenoniencs humoraux chez les Crustaces. L'adaptation chromatique et la croissance ovarienne chez la Crevette Leander serratus. *Ann. Inst. oceanogr.* Monaco **23**, 65.

Rangneker, P.V. 1955 The nutritive capacity of the blood of some representative crustaceans and molluscs. *J. Anim. Morph. Physiol.* **2**: 101-104.

Renaud, L. 1949 Le cycle des reserves organiques chez les Crustaces decapodes. *Ann. Inst. Oceanogr. Monaco*, **24**, 259.

Scheer, B. T. and Scheer, M.A.R. 1951 The hormonal regulation of metabolism in crustaceans. I—Blood sugar in spiny lobsters. *Physiol. Comp.* **2**, 193.

[Received for publication on 12th May, 1961]

OBSERVATIONS ON CERTAIN PROPERTIES OF THE LIVER LIPASE IN THE DEVELOPING CHICK

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LIVER is often considered a rich source for unspecific esterases. Even though there is some overlapping of substrate specificity between the unspecific esterases and lipase, it is generally believed that the enzyme hydrolyzing tributyrin is a true lipase (Dunkley and Smith, 1951). But according to Engelberg (1956), tributyrin can be hydrolyzed slightly by post-heparin lipoprotein lipase also. In the present study of the enzyme, the effect of some of the activators and inhibitors were tried in order to assess the propriety of regarding this enzyme as a lipase.

Material and Methods

White Leghorn chick embryos (15 to 18 days old) were carefully removed from the yolk and the liver sample was taken only from the left ventral lobe. Blood from the excised liver was blotted away with the aid of a filter paper. The tissue after having been weighed quickly was homogenized in distilled water in cold and diluted with cold distilled water so that each ml. of the homogenate contained 1.5 to 3 mg. wet weight of the tissue. The homogenate was centrifuged for 5 minutes at about 1500 × *g* and the supernatant was used as the enzyme solution. The enzyme activity was determined manometrically in a bicarbonate carbon dioxide buffer system of pH 7.4 at 37°C (adapted from Martin and Peers, 1953). An emulsion of tributyrin (4% v/v) in 0.0148 M sodium bicarbonate, prepared by shaking in a conical flask with a drop of "Tween 80", was used as the substrate. The reaction flask contained 1.5 ml. of 0.025 M sodium bicarbonate, 0.5 ml. of the substrate under test in concentrations to give the final concentrations tried and 0.5 ml. of the enzyme material in the main chamber and 0.5 ml. of the substrate in the side arm making a total volume of 3 ml. This gives a final concentration of 0.0148 M sodium bicarbonate. In the presence of 5% CO_2, this concentration of bicarbonate gives a pH of 7.4 (Umbreit *et al*, 1957). The flasks and manometers were gassed with a mixture of 5% CO_2 in nitrogen and equilibrated for 10 minutes in a water bath at 37°C. The substrate was then

tipped in and after another 3 minutes of equilibration, readings were taken at regular intervals for one hour. The manometers were shaken at 115-120 oscillations per minute with an amplitude of 5 cms. A control was run for each experiment in which 0.5 ml. of distilled water was added instead of the solution under test. Effects of sodium taurocholate, gall bladder bile, sodium fluoride, gum arabic, eserine sulphate, protamine sulphate and heparin were studied.

Results and Discussion

Effects of various substances

Sodium taurocholate:

Sodium taurocholate (Fisher Scientific Co.) at a concentration of 10^{-2} M was inhibitory. Sodium taurocholate activates pancreatic lipase (Nachlas and Seligman, 1949). However, according to Mattson and Beck (1956), bile salts have little effect on the hydrolysis of triglycerides. George and Scaria (1959) studied the properties of the pigeon pancreatic lipase and the pigeon breast muscle lipase and found that sodium taurocholate activates the pancreatic lipase but inhibits the pigeon muscle lipase. They also found that intermediary metabolites and ATP inhibit the pancreatic lipase, but activate the pigeon muscle lipase, and suggested that these two lipases are adapted for maximal activity in their own physiological environment in which they act.

Gall bladder bile:

Bile being the physiological environment of liver lipase, it was thought desirable to test the effect of fresh bile on the enzyme. Gall bladder from the same embryo from which the enzyme solution was prepared was broken into about 2 ml. of distilled water and used as the test substance. Since bile has some enzyme activity, it was determined separately and corrected for in the samples. The enzyme was activated about 30%.

Sodium flouride:

Sodium fluoride was found to inhibit the enzyme completely at a concentration of 0.2 M. Sodium fluoride at this concentration is shown to be a complete inhibitor for pancreatic lipase also (Hollet and Meng, 1956).

Gum arabic:

Gum arabic activated the enzyme slightly (3%) at a concentration of 0.5%. According to Fodor (1946) esterases are inhibited by gum arabic.

Eserine sulphate :

Eserine sulphate at a concentration of 10^{-5} M inhibited the enzyme by 30%. This substance is primarily noted for its inhibitory effect even in low concentrations on cholinesterases (Richter and Croft, 1942 ; Mendel and Rudney, 1943), and also the esterase activity of human serum (Nachlas and Seligman, 1949).

Heparin and Protamine sulphate :

Heparin at a concentration of 10 μg./ml. inhibited the enzyme by 5%. Protamine sulphate (0.005%) inhibited the enzyme by 35%. Protamine sulphate has been shown to inhibit the clearing activity of post-heparin plasma by 60% while the pancreatic lipase is not inhibited (Hollet and Meng, 1956). Heparin on the other hand is known to activate lipoprotein lipase.

Considering the action of sodium fluoride, gum arabic and bile, the liver lipase is more like the pancreatic lipase. The inhibition of liver lipase by sodium taurocholate could be explained. Sodium taurocholate at the same concentration (10^{-2} M) inhibits pigeon breast muscle lipase (George and Scaria, 1959), pigeon adipose tissue lipase (George and Eapen, 1960), chick brain lipase (George and Iype, 1961a) and pigeon heart and sheep heart muscle lipases (George and Iype, 1961b). In the light of these observations, it could be concluded that these lipases have some properties of their own not possessed by the pancreatic lipase.

However, the effect of eserine sulphate on the enzyme shows that this enzyme solution may contain some unspecific esterases also. Action of protamine sulphate indicates that some lipoprotein lipase is present in the enzyme preparation. But the result with heparin is just the opposite. These conflicting observations may be attributed to some traces of blood which might remain in the liver. It could also be that the concentrations tried might not act on the liver lipase as much as it does on the pancreatic lipase, this enzyme being more sensitive to some of the substances used.

The liver homogenate, it may be concluded, contains both unspecific esterases and a lipase. The problem is whether the simple esterases also could act on tributyrin and from the present work it seems probable that a small percentage of the enzyme activity is also due to the action of the unspecific esterases. Nevertheless, this overlapping in substrate specificity is none too significant, and much less, considerable. Hence this enzyme system could be considered, by and large, as that of a lipase.

Summary

The effect of some activators and inhibitors on the tributyrin-splitting enzyme of the developing chick liver were tried in order to assess the propriety of regarding this enzyme as a lipase.

Acknowledgement

I am indebted to Professor J. C. George, for the suggestion of the problem, guidance and for going through the manuscript.

References

Dunkley, W. L. and Smith, L. M 1951. Hydrolytic rancidity in milk. III. Tributyrinase determination as a measure of lipase. *J. Diary Sci.*, **34** : 935.

Engelberg, H. 1956. Human endogenous lipemia clearing activity. Studies of lipolysis and effect of inhibitors. *J. Biol. Chem.*, **222** : 601.

Fodor, P. J. 1946. Specific inhibition of esterases in ester hydrolyzing enzyme systems. *Nature*, **158** : 375.

George, J. C. and Eapen, J. 1960. A study of certain biochemical properties of the pigeon adipose tissue lipase. *J. Anim. Morph. Physiol.*, **7** : 60.

George, J. C. and Iype, P. T. 1961a. On the lipase activity in the developing chick brain. *J. Anim. Morph. Physiol.*, **8** :

George, J. C. and Iype, P. T. 1961b. Certain biochemical properties of the heart muscle lipase. *J. Anim. Morph. Physiol.*,

George, J. C. and Scaria, K. S. 1959. The pigeon breast muscle lipase. *J. Anim. Morph. Physiol.*, **6** : 55.

Hollet, C. and Meng, H. C. 1956. Comparison of fat emulsion clearing activities of post heparin plasma and pancreatic lipase. *Am. J. Physiol.*, **184** : 428.

Martin, H. F. and Peers, F. G. 1953. Oat lipase. *Biochem. J.*, **55** : 523.

Mattson, F. H. and Beck, L. W. 1956. The specificity of pancreatic lipase for the primary hydroxyl groups of glycerides. *J. Biol. Chem.*, **219** : 735.

Mendel, B. and Rudney, H. 1943. Studies on cholinesterase. I. Cholinesterase and pseudo-cholinesterase. *Biochem. J.*, **37** : 59.

Nachlas, M. M. and Seligman, A. M. 1949. Evidence for the specificity of esterase and lipase by the use of three chromogenic substrates. *J. Biol. Chem.*, **181** : 343.

Richter, D. and Croft, P. G. 1942. Blood esterases. *Biochem. J.*, **36** : 746.

Umbreit, W. W., Burris, R. H. and Stauffer, J. F. 1957. Manometric Techniques. Burgess Publishing Co., Minneapolis.

[Received for publication on 24th March, 1961]

A QUANTITATIVE STUDY OF THE DISTRIBUTION PATTERN OF IRON IN THE RED AND WHITE FIBRES OF THE PIGEON BREAST MUSCLE

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George and Naik (1959) studied the fibre architecture of the *Pectoralis major* muscle of the pigeon in detail and showed that the least number of broad fibres is found in the fasciculi situated in the middle of the dorso-ventral axis of the muscle and that the number tends to increase above or below this mark, reaching the maximum in the most superficial and in the deepest layers of the muscle. Based on this finding the distribution of succinic dehydrogenase activity was studied quantitatively in the different layers of this muscle by recording the distribution pattern of the narrow red and the broad white fibres side by side (George and Talesara, 1960). It was found that the main bulk of the enzyme activity is confined to the narrow red fibres. In a further study (George and Talesara, 1961a) the extent of the activity of some oxidizing enzymes and a hydrolyzing enzyme, lipase, were assessed quantitatively in the different layers of the pigeon breast muscle and the respective enzyme concentrations in the individual red and white fibres were estimated using the statistical derivation employed as reported earlier (George and Talesara, 1960). Such a procedure was adopted because it is extremely difficult to separate out the fibre types of a mixed muscle such as this and then estimate directly the various biochemical constituents. Moreover, there are certain labile constituents which could neither be studied histochemically nor biochemically and could not be estimated even if the separation of the fibre types was realized. Hence the present investigation deals with a study undertaken to assess quantitatively the iron content of the red as well as the white fibres of the pigeon breast muscle, by using an indirect method as mentioned above.

Material and Methods

The *pectoralis major* muscle of the well fed and fully grown, normal laboratory pigeons (*Columba livia*), was used throughout the study.

For each experiment a bird was decapitated and the blood completely drained off. A muscle strip of nearly 2 square cm. area cut along the direction of the fibres, was removed from the middle of the body of the pectoralis in its entire thickness. Another piece with exactly the same topography was cut quickly from the muscle of the other side. In all sets of experiments the muscle strip removed was only from this particular region. In each set of experiments one such piece of the muscle was used for the estimation of iron and the other for recording the distribution pattern of the broad and narrow fibres per square mm. at different depths of the muscle. Slices of the desired thickness were cut on a freezing microtome and the relative distribution of the fibre types per square mm. in the different depths was assessed according to the procedure already referred to (George and Talesara, 1960). The only change adopted in the present study that each of the slices cut, was transferred immediately to weighing bottles and dried in an air oven at 100°C till a constant dry weight was obtained. In some cases, however, it was preferred to pool slices from 3 or 4 muscle pieces taken from different pigeons. Thus after obtaining the constant dry weight of the muscle slices, the iron content was determined by Kennedy's method as described in Hawk *et al* (1954). The muscle was digested in concentrated H_2SO_4 in the presence of perchloric acid and potassium thiocyanate was added to a part of this. The colour developed was extracted with amyl alcohol and the intensity was read on a Klett-Summerson photoelectric colorimeter.

Results and Discussion

The iron content was found to vary at different depths of the muscle as is shown in Table 1. The iron content was also determined in the 0.5 mm. thickness of the most superficial layers starting from the ventral face of the muscle where the broad fibres are relatively much more concentrated than in the one-mm. thick layer (Table 1). The relative distribution pattern of the broad and narrow fibres at a particular depth or layer is also recorded in the same table. These data clearly indicate that the variation in the chemical content is in accordance with the variation in the ratio of the two types of fibres in their distribution pattern in the different layers. The same conclusion was arrived at in the case of of metabolites like fat and glycogen (George and Naik, 1959) and oxidative enzymes and a lipase (George and Talesara, 1960, 1961a).

TABLE I

Showing the distribution pattern of iron in the different layers of the *pectoralis major* muscle of pigeon and their relation to the number of muscle fibres (The figures indicate the average values of five sets of experiments in each case)

Expt.	Depths of the muscle in mm. starting from the superficial side (ventral face)	No. of fibres per square mm.		Iron in mg. per 100 gm. dry muscle
		Broad	Narrow	
1	0-0.5	119 ± 11	214	14.64
	0.5-1.0	88 ± 9	385	23.20
	0-1	102 ± 8	301	19.53
	1-2	78 ± 6	471	26.56
2	2-3	56 ± 7	567	30 74
	3-4	44 ± 3	640	33.90
	4-5	34 ± 6	696	38.06

The first observation which can be made from this study is that unless the ratio of the distribution pattern of the different type of fibres that constitute the muscle under investigation is taken into consideration while assessing its biochemical constituents no accurate values could be obtained as have been the case in the numerous investigations hitherto made. The average values obtaincd in the earlier studies might give a broad average for the muscle as a whole which certainly does not constitute the true picture of the muscle and its cellular components.

The results obtained for the different layers in this study indicate that with the increase in the number of narrow over the broad fibres in a particular layer the iron content also increases. It is also interesting to note that the iron content is maximum in the layer taken from the middle of the dorso-ventral axis where the number of broad fibres is the least. Iron content tends to decrease above this mark and is the least in the most superficial layer where the number of broad fibres is maximum. A graphical presentation (Fig. 1) of the distribution of narrow and broad fibres per square mm. in a particular layer against the values for iron show that the major amount of iron is confined to the narrow red fibres whereas the broad white fibres have considerably less.

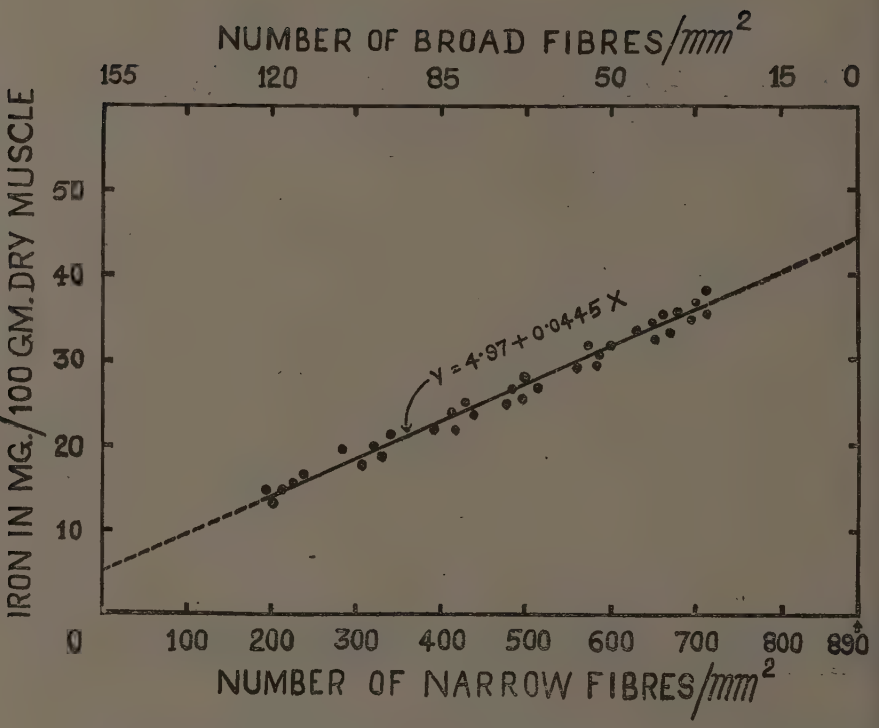

Fig. 1 Graph showing the relation between the number of narrow and broad fibres per square mm, and the iron content as obtained at different depths of the *pectoralis major* muscle of the pigeon.

George and Naik (1959) showed that per unit area of cross-section of the muscle at any particular depth, the number of broad fibres is inversely proportional to the number of narrow fibres and derived the formula

$$Y = -5.75\ X + 890.01,$$

(where Y stands for the number of narrow fibres and X for the number of broad fibres). It means that when X is zero the total number of narrow fibres per square mm. area would be 890 *i.e.* all narrow fibres and when Y is zero, the total number of broad fibres per square mm. area nearly 155 *i.e.* all broad fibres. It should be mentioned, however, that this is merely a theoritical assumption and such a condition never exists in this muscle in any region. However, considering the nature of the regression line (Fig. 1), which shows a linear pattern, it is possible to

derive the iron content of the individual fibre types, when this line is extened on either sides as shown by broken lines (Fig. 1).

The values so derived for the individual fibre types are shown separately in Table 2. It should be noted, however, that the values derived by this indirect method may not be absolute, neverthless, does provide a reasonably accurate method for a quantitative determination of the various constituents specially certain labile constituents and enzymatic activities which cannot be revealed either histochemically or biochemically in the two fibre types of this muscle without isolating them. On this basis the values of certain oxidizing enzymes and a lipase for the red and white fibres respectively, also have been derived (George and Talesara, 1961a).

TABLE II

Iron content of the red as well as the white fibres of the pigeon *pectoralis major* muscle as derived from the equation of the regression line

Muscle	Iron in mg./100 gm. dry muscle
Red	44·57
White	4·97

The considerably higher content of iron in the narrow red fibres of the pigeon breast muscle indicate that these fibres are rich in myoglobin. Higher concentrations of this pigment are known to be present in the muscles having a high aerobic metabolism and the strong affinity of myoglobin for oxygen enables it to act as an intramuscular oxygen store for tissue oxidation. Lawrie (1952, 1953a and 1953b) in his biochemical studies on different types of muscles has shown that there is a parallelism in muscles between the percentage of myoglobin, the activity of cytochrome oxidase and a high capacity for aerobic synthesis of high energy phosphates. Thus the very low concentrations of oxidizing enzymes (George and Talesara, 1960, 1961a, 1961b and 1961c) and myoglobin as indicated by the low iron content in the broad white fibres do indicate a poorly developed respiratory metabolism in them as against the narrow red fibres of the pigeon breast muscle.

Summary

Iron content was determined in the different layers of the pigeon breast muscle on the basis of the distribution pattern of the broad and narrow fibres in this muscle. The variation in the iron content in the different layers was found to be due to the variation in the ratio of the distribution pattern of the two types of fibres. Iron content was found to be highest in the interior of the muscle and lowest in the superficial layers. From the statistical analysis of the results obtained it was possible to derive the iron content of the two fibre types, without isolating them. The possible significance associated with the difference in the iron content of the two types of fibres in this muscle is discussed.

Acknowledgement

I am grateful to Professor J. C. George, for the suggestion of the problem, guidance and for going through the manuscript.

References

George, J. C. and Naik, R. M. 1959. Studies on the structure and physiology of the flight muscles of birds. 4. Observations on the fibre architecture of the *pectoralis major* muscle of the pigeon. *Biol. Bull.* **116** : 239.

George, J. C. and Talesara, C. L. 1960. Studies on the structure and physiology of the flight muscles of birds. 9. A quantitative study of the distribution pattern of succinic dehydrogenase in the *pectoralis major* muscle of the pigeon *Ibid.*, **118**, 262.

George, J. C. and Talesara, C. L. 1961a. A quantitative study of the distribution pattern of certain oxidizing enzymes and a lipase in the red and white fibres of the pigeon breast muscle. Accepted for publication.

George, J. C. and Talesara, C. L. 1961b. Histochemical observations on the succinic dehydrogenase and cytochrome oxidase activity in pigeon breast-muscle. *Quart. J. micr. Sci.* **102**, 131.

George, J. C. and Talesara, C. L. 1961c. Histochemical demonstration of certain DPN-linked dehydrogenases and aldolase in the red and white fibres of the pigeon breast muscle. Accepted for publication.

Hawk, P. B., Oser, B. L. and Summerson, W. H. 1954. Practical Physiological Chemistry, Mc Graw-Hill Company INC., New York.

Lawrie, R. A. 1952 Biochemical differences between red and white muscle. *Nature*, **170**, 122.

Lawrie, R. A. 1953a. The activity of the cytochrome system in muscle and its relation to myoglobin. *Biochem. J.*, **55**, 298.

Lawrie, R A. 1953b. A relation of energy rich phosphate in muscle to myoglobin and to cytochrome oxidase activity. *Ibid.*, **55**, 305.

[Received for publication on 18th February, 1961.]

ACTIVATION OF PHOSPHOMONOESTERASES OF BULL SEMEN IN MILK DILUENT: PASTEURISED WHOLE MILK HEATED TO 90°C FOR TEN MINUTES

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PHOSPHOMONOESTERASE I or alkaline phosphatase is very widely distributed in biological systems. It is found in all animal cells with few exceptions. The optimum pH of this enzyme is known to be between 9.2 and 9.6 and slightly lower in the case of crude preparations. The principal activators of alkaline phosphatase are the divalent cations, the most important of these being Mg^{++}. Insufficiently purified preparations exhibit a progressive activation by Mg^{++} in proportion to its concentration up to an optimum level of M/200 in most cases and beyond that level this effect is less pronounced (Erdtmann, 1927, Erdtmann, 1928). Amino acids, Alanine and higher homologues among others increase the effect of the cations (Hove *et al* 1940; Roche *et al* 1944). Mg^{++} is not the only activating cation, it may be replaced by Mn^{++}, Zn^{++}, Co^{++}, (Bamann, 1940; Massart *et al* 1940, and Bodansky 1946) but at optimum concentration characteristic of each of them. It is, however, much lower for zinc salts. Fe^{++}, Ni^{++} and under certain conditions Ca^{++}, are less effective activators. Above an optimum level aminoacids become inhibitory (Roche *et al* 1944; Bodansky 1946).

The most important inhibitors are phosphate and arsenate ions (Roche *et al* 1943) and various metal forming compounds. Among these are cyanides. Sulfhydryl compounds particularly cystein are powerful inhibitors (Martland *et al* 1927; Waldschmidt *et al* 1933). The same is true of hydrogen sulphide while fluorides and pyrophosphates inhibit to a much lesser degree under special conditions (Nguyen-Van Thoai *et al* 1947). Oxalates inhibit slightly (Belfanti 1935).

Phosphomonoesterases II or acid phosphatase is also widely distributed in nature. The pH optimum of this enzyme varies from 5.2 to 5.4. The most characteristic modifying factors are inhibitors. Besides phosphate and arsenate ions which are effective against all phosphoesterases, certain ions inhibit acid phosphatase even at low concentrations. F^- is

[J. Anim. Morph. Physiol., Vol. 8, No. 2, pp. 155 159, 1961]

a strong inhibitor at a concentration of M/1000 to M/5000 (Sizer 1942 ; Pearlmann *et al* 1942). Conversely Mg^{++} and divalent cations, sulfhydryl compounds, amino acids and cyanides have no effect on acid phosphatase Bamann *et al* 1934). Oxalates are strong inhibitors (Belfanti 1935).

Materials and Methods

The work comprises data from ten dairy bulls maintained by The Department of Dairy and Animal Husbandry, Oregon State University, Corvallis, Oregon. Semen was collected once weekly as per the method described by Lambert and Mackenzie (1940) and split samples diluted with physiological saline and pasteurized and homogenized milk heated to 90°c for 10 minutes. The semen so diluted was kept in the refrigerator till enzyme determinations were carried out. Simultaneous enzyme determinations were carried out as per the method described by Hawk *et al* (1951). At least fifteen such determinations were undertaken for each animal with milk and saline diluted semen. The enzyme determinations were carried out in triplicate to increase the accuracy of the results obtained. The bulls from which semen was collected belonged to three breeds, *viz.* Holstein-Friesian, Brown swiss, and Jersey.

TABLE I

Mean alkaline and acid phosphatase values in split bull semen samples diluted with heated milk and saline expressed in Bodansky units/100 ml. semen

Animal No.	'Saline diluted semen'		Milk diluted semen	
	'Alkaline'	Acid	'Alkaline'	Acid
1	327	101	462	120
2	282	122	384	124
3	233	96	361	90
4	430	86	560	104
5	760	110	940	108
6	460	90	538	105
7	430	85	630	98
8	224	73	320	86
9	640	115	860	112
10	423	92	575	93

TABLE II

The Alkaline enzyme activity of split semen samples diluted with saline and heated milk subjected to analysis of variance

Treatment	Sum of squares	d.f.	Mean square	F	F 05	F 01
Between treatment	.26	1	.26	7.22**	3.84	6.64
Error..	10.69	298	0.036	—	—	—
Total ..	10.95	299	—	—	—	—

** Significant at 1% level.

TABLE III

Acid enzyme activity of split semen samples diluted with saline and heated milk subjected to analysis of variance

Treatment	Sum of squares	d.f.	Mean square	F	F 05	F 01
Between treatment	.052	1	.052	1.68	3.84	6.64
Error..	9.32	298	.031	—	—	—
Total ..	9.84	299	—	—	—	—

Results and Discussion

The table I clearly shows that milk activates the semen alkaline enzyme in all the ten bulls on experiment. The data obtained when subjected to analysis of variance, it is found to be significant at 1% for alkaline enzyme and not significant in case of acid enzyme. This shows that milk does not activate semen acid phosphatase but activates alkaline enzyme to a considerable degree. The milk heated for 90°C for 10 minutes used for dilution of semen showed no enzyme activity. This was further ascertained by simultaneous enzyme determinations of heated milk. The results are not unexpected as milk contains all essential amino acids, including alanine and several minerals like Mg, Ca which activates the semen alkaline phosphatase.. This perhaps is the reason for increased enzyme activity when heated milk is used as a diluent. By heating milk to 90°C for ten minutes the sulfhydryl group is precipitated and this in turn prevent

inhibition of the alkaline enzyme activity (Roche *et al* 1943). The high level of phosphatase in semen indicates that the enzyme is in some way related to sperm metabolism. It shows why heated milk is perhaps successfully used as a semen diluent. None of the minerals have any effect on acid phosphatase which explains why acid phosphatase levels remain unaffected when milk is used as a diluent.

Summary

Bull semen when diluted with pasteurized whole milk heated to 90°C for ten mimutes was found to activate semen alkaline phosphatase and not acid phosphatase which was significant at 1% level when compared to semen simultaneously diluted with physiological saline.

Acknowledgements

The study was completed at Oregon State University while the author was working with Professor F. F. Mckenzie, Department of Animal Husbandry and is a part of the thesis submitted in partial fulfilment of the degree of Doctor of Philosophy. The Author is grateful to Dr. F. F. Mckenzie and Professor Hugo Kruegar for guidance and suggestions.

References

Bamann, E. and Diederichs, K. 1934. Phosphatases III. The seperation of the two isodynamic phosphoesterases of animal organs through selective inactivation. *Ber.* **67B**, 299.

Bamann, E. and Diederichs, K. 1935. Phosphatases IV. Separation of esterase from the liver phosphoesterase active in acid media. *Ber.* **68B**, 6.

Bamann, E. 1940. Naturwissenschaften **23**, 142.

Belfanti, S. *et al* 1935. The phosphatases II. Inactivation and reactivation of the phosphatases of animal organs. *Biochem. J.*, **29**, 842.

Bodansky, O. 1946. Mechanism of inhibition of phosphatase activity by glycine. *J. Biol. Chem*, **165**, 605.

Eapen, K. J. 1959. The influence of dietary carotene levels on semen phosphatases in farm animals. Diss. Abstr. 19, 3070.

Eapen, K. J. 1961. Influence of dietary carotene levels on semen phosphatases in dairy bulls. *J. Anim. Morph. physiol.* **8**, 53.

Erdtmann, H. 1927. Glycero phosphate clavage by kidney phosphatase and its activation. *Z. Physiol. Chem*, **172**, 182.

Erdtmann, H. 1928. Kidney phosphatase and its activation II. *Z. Physiol. Chem.*, **177**, 211.

Hove, E. *et al* 1940. Effect of zinc on alkaline phosphatase. *J. Biol. chem.*, **134**, 425.

Hawk, P. B. *et al* 1951. Practical physiological chemistry 12th Ed., New York. Blackiston.

Lambert, W. V. *et al* 1940. Artificial insemination in live stock breeding, U.S.D.A, Circular No. 567.

Martland, M. and Robison, R. 1927. The possible significance of hexose phosphoric esters in ossification. Part VII. The bone phosphatase. *Biochem. J*, **21**, 665.

Massart, L. *et al* 1940. Activation and inhibition of phosphatases. Naturwissenschaften. **28**, 143.

Moog, F. 1946. The physiological significance of phosphomonoesterases. *Cambridge Pil. Soe., Biol. Rev.* **21**, 41.

Newyen-Van-Thoai *et al.* 1947. Mechanism of the inhibition of alkaline phosphatase by fluoride, phosphate and pyrophosphate ions. *Compt. Rend. Soc. Biol.*, 140, 149.

Perlmann, G. E. and Ferry, R. M. 1942. A note on the separation of kidney phosphatases. *J. Biol. chem.*, **142**, 573.

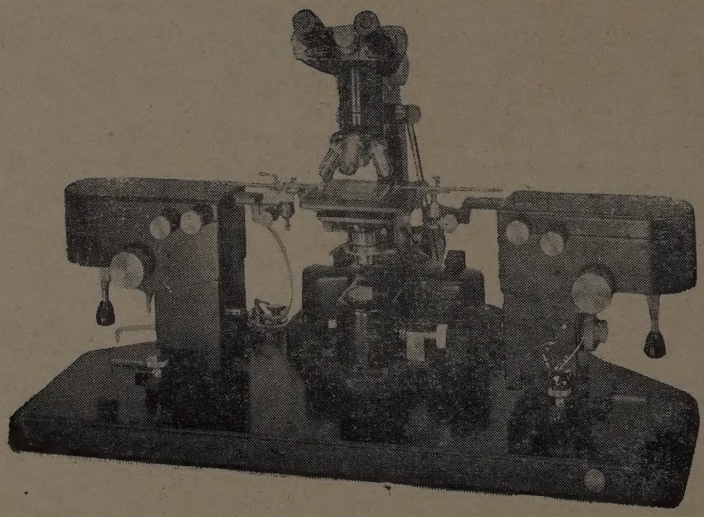
Roche, J. *et al* 1944. Coupled action of cysteine and ferrous ions on phosphatases. *Comt. Rend. Soc. Biol.*, **137**, 541.

Sizer, I. W. 1942. The activation of certain oxidants and reductants upon the activity of bovine phosphatase. *J. Biol. Chem.*, **145**, 405.

Sumner, J. B. and MyrBack, K. 1950. The Enzymes, Vol. I Part I. Academic Press. Inc. New York.

Waldschmidt, L. *et al* 1933. The influence of sulfhydryl compounds on enzymic processes. *Z. physiol. chem.*, **214**, 75.

[Received for publication on 13th January, 1961]



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Printed by Ramanlal J. Patel, Manager, The M. S. University of Baroda Press (Sadhana Press), near Palace Gate, Palace Road, Baroda, and edited and published by Dr. J. C. George, for the Society of Animal Morphologists and Physiologists, at Dept. of Zoology, Faculty of Science, Baroda 1. Dec. 1961.